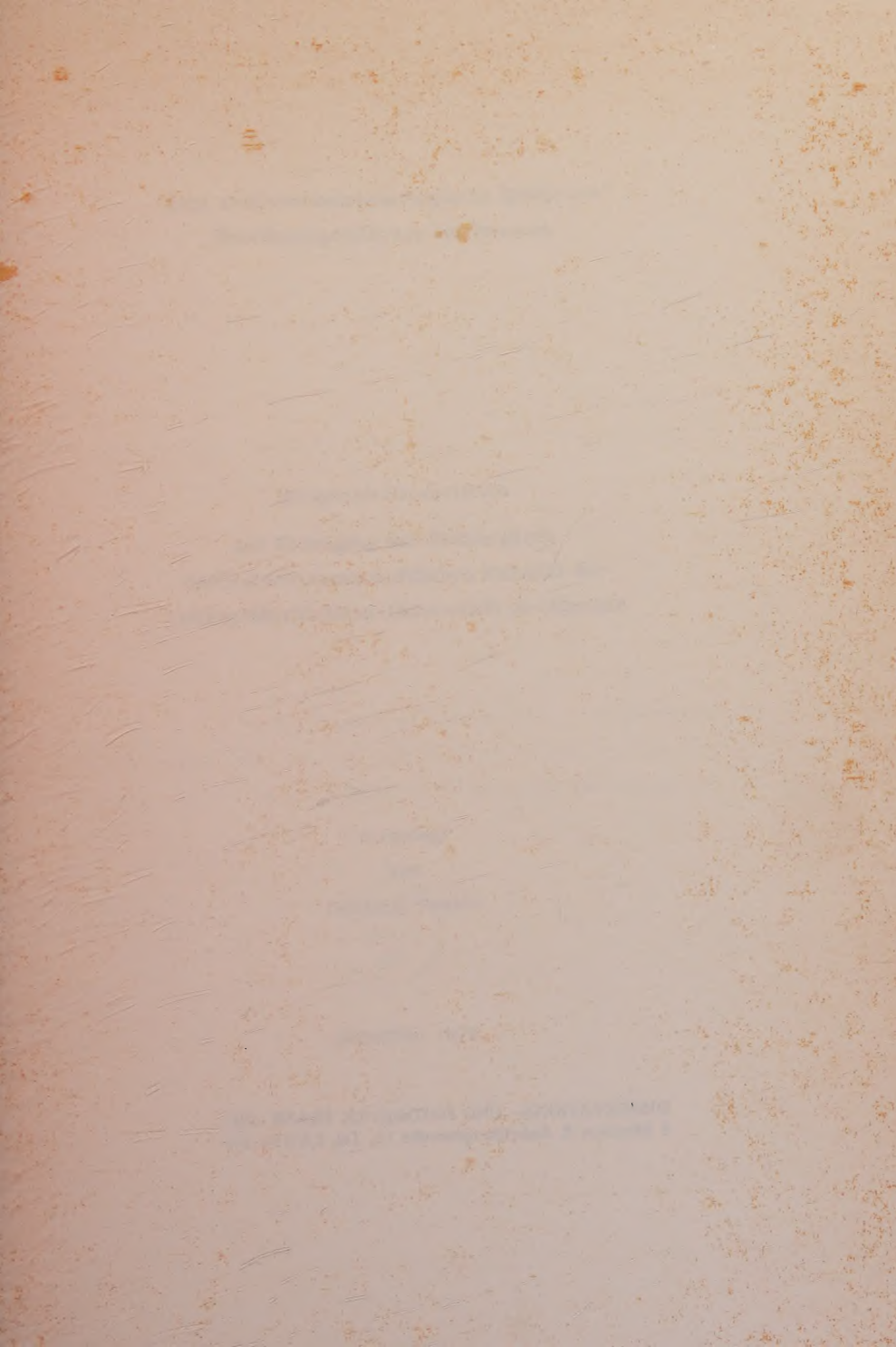


**Eine stoffwechselphysiologische Studie der
Überflutungstoleranz bei Bäumen**

Gertrud Vester

1972



DISSERTATIONS- UND FOTODRUCK FRANK oHG
8 München 2, Gabelsbergerstraße 15, Tel. 2 8 0 9 0 9 0

Eine stoffwechselphysiologische Studie der
Überflutungstoleranz bei Bäumen

Inaugural-Dissertation
zur Erlangung der Doktorwürde
der Naturwissenschaftlichen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt
von
Gertrud Vester

München 1972

Berichterstatter: Professor Dr. Otto Kandler

Tag der mündlichen Prüfung: 14.11.1972



A Metabolic Study of Flooding Tolerance in Trees.

Gertrud Vester

A thesis submitted for the degree Doktor der Naturwissenschaften,
University of Munich, October, 1972.

Curriculum vitae

Date and place of birth: 11th November, 1943, Munich.

Parents: Hugo and Hertha Vester (née Staedel)

Education: 1964 - left school after passing University entrance examination.

1964-69 - studied at University of Munich and passed in Autumn 1969 the State Examination in Chemistry and Biology.

Research: 1970-72. Research assistant in the Botany Department of the University of St. Andrews.

CERTIFICATE

I hereby certify that Gertrud Vester has been engaged upon research work from January 1970 onwards under my supervision to prepare the accompanying thesis for the degree of Doktor der Naturwissenschaften in Munich.

R. M. M. Crawford

R.M.M. Crawford
(Reader in Botany)

St. Andrews,
October, 1972.

DECLARATION

I hereby declare that the following thesis is based upon work done by me, that the thesis is my own composition and that it has not been previously presented for a higher degree.

The research work was carried out in the department of Botany, University of St. Andrews, under the supervision of Dr. R.M.M. Crawford.

James D. ...

St. Andrews

October, 1972.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisors, Professor Dr. O. Kandler and Dr. R.M.M. Crawford, for their encouragement and advice over the past three years. In particular, I wish to thank Dr. R.M.M. Crawford for his great patience and help in solving the problems which arose in the course of my research work in St. Andrews and those which I had to overcome through living in a foreign country.

I also wish to thank Professor J.A. Macdonald, in whose department the work was carried out, other staff and students, especially Mr. R. Stephenson for photographic work, Mrs. R. Bevan for assistance with drawing the Figures, Mrs. M. Dobson for typing the thesis and Mr. W.I.P. Hazlett for teaching me to write good English.

The work was carried out during the tenure of an N.E.R.C. Research Studentship.

CONTENTS

	Page
Introduction	1 - 4
Methods	4 - 9
Plant Material	10 - 11
Chapter I : Shoot and root morphology in relation to flooding-tolerance	12 - 28
1. Shoot growth	12 - 15
2. Temporary and permanent dormancy	15 - 17
3. Adventitious roots	17 - 20
4. Shoot height and age	20 - 22
5. Shoot growth in glasshouse and experimental garden	22 - 25
6. Root:Shoot Ratio	25 - 27
Conclusion	27 - 28
Chapter II : Carbon dioxide fixation and evolution from roots in relation to flooding tolerance	29 - 42
1. Unflooded tree seedlings	30 - 32
2. Effect of short period of submerged soil conditions	33 - 34
3. Long-period flooding	34 - 40
Conclusion	41 - 42
Chapter III : Distribution of carbon dioxide fixation products in roots in relation to flooding tolerance	43 - 69
1. The effect of oxygen supply on the distribution of the $C^{14}O_2$ -fixation products in roots of unflooded tree seedlings	44 - 47
2. Effect of water-logged soil conditions on the distribution of the $C^{14}O_2$ -fixation products of roots	47 - 55
3. Effect of additional nutrients on the distribution of $C^{14}O_2$ -fixation products in roots in relation to flooding tolerance	56 - 64
Conclusion	64 - 69
Chapter IV : Quantitative changes in certain tree root metabolites in relation to flooding tolerance.	70 - 77
1. Changes in the fructose, glucose and sucrose content of roots due to experimental flooding	70 - 71

CONTENTS (continued)

Chapter IV

2. Ethanol and malic acid contents of birch sap in relation to dry and wet habitats	72 - 73
3. Glycerol and malic acid contents of <u>Alnus incana</u> roots in relation to experimental flooding	73 - 74
4. Internal gases in shoot and root of tree seedlings in relation to flooding tolerance	74 - 76
Conclusion	76 - 77
Discussion	78 - 91
Summary	92 - 94
References	95 - 99
Zusammenfassung	i - ii

INTRODUCTION

The demand for land under the present conditions of industrial growth in Western Europe, has caused a re-appraisal of the economics of afforestation and timber production. According to Barthelmess (1972), the value of forests in highly industrialised societies, such as Germany, lies in their amenity and recreational uses. In terms of pure economics, however, while the condition of the land may be excellent for tree-growth, its potential value for other purposes renders forestry uneconomic. For this reason timber production in Europe will be limited in future to the more northern areas where the demand for land is less. However, in these northern areas (the native boreal forest zone), where cooler climatic conditions prevail, the absence of a potential water deficit leads to the production of saturated soils. Under such conditions, drainage removes only gravitational water and the soil remains at field capacity for most of the year. The boreal zone as a whole is characterized by large areas of bogs, muskegs and peat soils. Although in eastern Europe many areas remain where the natural forest vegetation has not been removed, in the west the situation is quite different. Until recent times, large rural populations and later, sheep farming, led to extensive deafforestation of very large areas. Such a site is illustrated in Photograph A showing a typical treeless landscape in Scotland. The removal of the tree cover reduces the transpiration and evaporation that takes place from the vegetation surface, and increases the water-saturation of the soil. Thus when

Photograph A.

A typical treeless landscape in Scotland. Panorama view of Ben Lawers, Perthshire.



A natural *Pinus sylvestris* forest with Juniper and heather. Rothiemurchus Nature Reserve, Scotland.



2.

re-afforestation is contemplated, it is essential that tree varieties be chosen that are resistant to waterlogged conditions.

The problem of selecting trees with flooding-tolerance is said to pose biological and practical difficulties. The first symptom which the forester sees is usually the premature wind-throw of large blocks of plantations. The saturation of the lower soil-level can prevent the development of anchoring roots in certain susceptible strains of trees. It is the recognition of these susceptible varieties which represents serious practical problems. It is usually not until the tree has obtained a considerable size (some 15 to 20 years after planting) that wind-throw symptoms due to waterlogging become apparent. As an empirical test for flood-tolerant strains, it has been practised by the British Forestry Commission and consists of measuring the force that is necessary to pull down a variety of trees growing in different sites. The greater the force needed to pull down a tree, the better the anchoring development, and presumably also the flooding-tolerance of the roots in a wet site. The disadvantages of this method are threefold. First, the results of flooding-tolerance are not available till after 15 to 20 years after the election of the strains of the provenances. Secondly, it has to be assumed that the soil conditions have not altered during the test period, and poor development of roots when encountered is due to waterlogged conditions. Thirdly, the testing of the material also destroys it as a source of seed.

3.

These difficulties, together with the time and costs of these methods, prompted the Forestry Commission to ask the St. Andrews group if it was possible to make a biochemical study of tree-seedlings which could be used to predict the flooding-tolerance of mature trees. This thesis therefore is the result of several years of enquiry into this problem by the St. Andrews Botany Department and the Forestry Commission.

The work carried out on flooding-tolerance in trees so far has been of a practical nature using transplants and varying draining systems. A review of these studies has been made by Gill (1970). From this work it is clear that not only different species vary in their flooding-tolerance but also different strains within the species. However, apart from preliminary experiments in the U.S.S.R. and U.S.A. (Čirkova, 1968; Dubinina, 1962; Hook et al., 1971; and Veretánnikov, 1968), little work has been done on the physiological differences of flood-tolerant and intolerant trees. The question examined in this thesis is whether physiological differences do exist and can be used as a practical prognosis of flooding-tolerance under field condition.

Plan of investigation

The main question to be asked concerns the nature of flooding-tolerance. Is the ability to maintain a living root-system in a water-saturated soil due to metabolic properties, or to morphological adaptations facilitating root-shoot gases exchange? It may be that a combination of these factors has to be taken into account.

4.

The thesis is, therefore, divided into a number of sections in which (a) the use of seedlings is compared with larger trees, (b) the metabolism of tolerant and intolerant strains is investigated, and (c) the diffusion of organic gases from the root to the shoot is analysed. In conjunction with all these physiological studies, the growth of the tree-seedlings is examined closely under varying conditions. Thus it is hoped that as accurate a prediction as possible of growth, form, and function in trees in relation to flooding-tolerance is presented.

METHODS

1. Hoagland's solution:

as described by Thomas, Ranson and Richardson (1956).

2. Preparation and extraction of root-material for biological investigations:

(i) C¹⁴O₂-fixation experiments

Only chilled liquids were used for the root-cleaning procedure. The root system was gently washed free from peat and sand, at first in tap-water and then in distilled water. The surface of the roots was sterilized with 0.02 % mercuric chloride (Crawford, 1966) and subsequently rinsed in sterile water several times and blotted damp-dry with paper tissue.

The exposure to C¹⁴O₂ was done in Warburg flasks as described by Peel (1953). The exposure medium was 3.0 ml 0.15 M phosphate buffer at pH 5.6 and 0.5 ml of 0.31 M NaCO₃ containing 50 µl of C¹⁴. During the exposure to C¹⁴ for one hour, manometer readings were taken every ten minutes. At the end of this period, the roots were extracted three times with 80 percent and three times with 50 percent ethanol.

(ii) Glycerol and malate determination

Liquid nitrogen was applied to aid maceration of the roots. The deproteinisation and neutralisation was done according to Bergmeyer (1963), page 213.

(iii) Carbohydrate determination

After being washed with chilled tap water and distilled water, and determination of fresh-weight, the roots were cut into approximately 2 cm lengths and extracted three times with 80 percent and three times with 50 percent ethanol.

3. Collection, deproteinisation and neutralisation of rising birch sap (cf. Photograph B):

A hole was bored into the tree trunk and a plastic tube pressed into it to let the sap drip into a bottle containing 6% perchloric acid. The deproteinisation and neutralisation was performed as described by Bergmeyer (1963), page 330.

4. Separation of the labelled $C^{14}O_2$ -fixation products:

Approximately 500 counts/min. of labelled extract was put on a 65 x 65 cms Whatman paper No.4. If the extract was weakly labelled, a smaller quantity had to be taken to avoid overloading of the chromatogram. As a marker to aid the subsequent identification of the labelled substances, 20 μ g of one of the following mixtures of amino acids were added:

- (i) Asparagine, alanine, valine, leucine;
- (ii) Asparagine, glutamine, aminobutyric acid, leucine or
- (iii) Asparagine, alanine, glycine, tyrosine.

The chromatograms were run in two dimensions as described by Benson et al. (1950). That is to say, for the long direction water saturated phenol was used (24-26 hours) and for the short direction, butanol (750 ccs and 50 ccs distilled water) and propionic acid (352 ccs and 448 ccs) were applied (12-16 hours).

5. Exposure of chromatograms and counting:

The dried chromatograms were placed on X-ray films for one to two months, according to the applied radio activity of the origin. Photographs C and D are examples of the developed X-ray films.

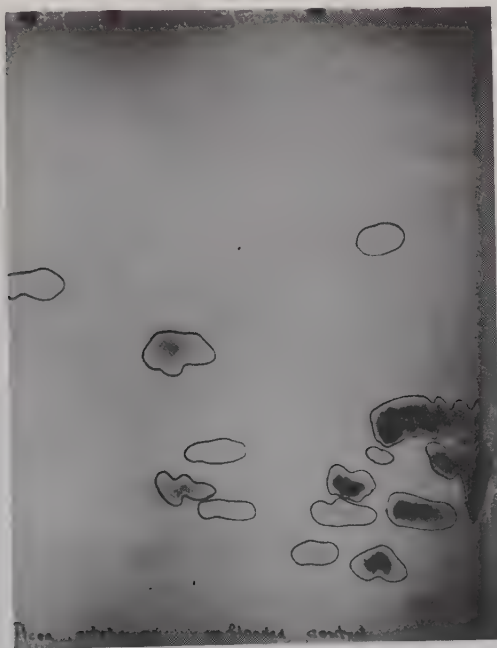
Photograph B.

Collection of birch sap.



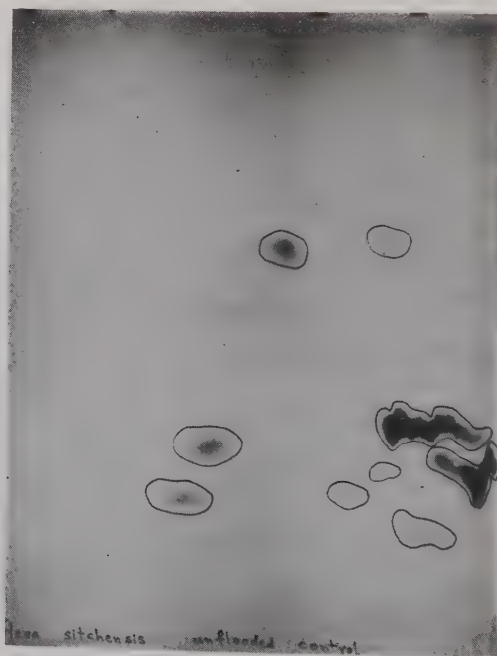
Photograph C.

Radiograph of the ethanol extract of Picea sitchensis seedlings after their roots had fixed $C^{14}O_2$ under air for one hour. The paper chromatogram from which this radio-autograph was prepared was developed horizontally in phenol and vertically in butanol-propionic acid - water solvent (Benson et al., 1950). Malic, citric, glutamic, aspartic acids and UMP are the major labelled products.



Photograph D.

Radiograph of the ethanol extract of Picea sitchensis seedlings after their roots had fixed $C^{14}O_2$ under nitrogen for one hour. The paper chromatogram was prepared as described in Photograph C. Malic, citric and succinic acids are the major labelled products.



7.

The regions of activity were marked and cut out. The activity of each "spot" was determined by using a methane flow-counter for ten minutes, counting each "spot" three times.

6. Identification of labelled metabolites:

Carrying amino acids as markers, the chromatograms, without the radioactive "spots", were sprayed with ninhydrin solution. The comparison of the radiograms and sprayed chromatograms with data from the literature provided a good basis for accurate identification tests. Some "spots" of each guessed substance, with the relatively highest counts per minute, were eluted with water, dried and used for the following identification tests.

(i) Co-chromatograms

Organic acids. Solvent by Higgins and von Brand (1966): Propanol/eucalyptol/formic acid/water in the ratio 50/50/20/5. Indicator reagent: Aniline - glucose as described by Carles et al. (1958).

Amino acids. Solvents by Hausmann (1952):

1. sec-butanol and 3% ammonia in the ratio 150/60
 2. sec-butanol, formic acid and water in the ratio 150/30/20.
- Indicator reagent: Commercial preparation of ninhydrin spray. As the activity of most "spots" was low, the co-chromatograms were exposed for 2 months to X-ray films before they were sprayed with the indicator reagent.

(ii) Mass spectroscopy of the organic acids.

The residue of the eluted substance was dissolved in water-free methanol and with the aid of a mass spectroscope, compared with a standard.

(iii) Enzymatic determinations

Glutamic acid was measured with glutamic dehydrogenase and malate with malate dehydrogenase (Bergmeyer, 1963, pages 384 and 328).

(iv) Sugars and UMP by means of gas-liquid chromatography

The preparation of the trimethyl silyl ether derivatives was performed as described by Sweeley et al. (1963).

7. Ethanol determination:

This was done by means of difference spectroscopy using alcohol dehydrogenase (Bergmeyer, 1963, p.285). The modification of applying difference spectroscopy was found necessary, because of the very low ethanol concentrations of rising birch-sap.

8. Malate determination - see 6.(iii).

9. Glycerol determination:

A commercial preparation of buffer and enzymes was used, from Boehringer/Mannheim, including glycerol kinase, pyruvate kinase and lactate dehydrogenase.

10. Determination of fructose, glucose and sucrose

The identity was checked and the concentrations determined by means of injecting the standards of trimethyl silyl ethers (Sweeley et al. 1963),

11. Extraction and determination of internal gases:

The extraction of the internal gases, methane, ethylene and acetylene was performed as described for acetylene by Beyer and Morgan (1970). The only alterations were that (i) a solution of surfactant to reduce adhesion of air bubbles

9.

was not used, as it was found to be unnecessary and (ii) a constant vacuum of 100 mm of Hg for two minutes was not applied, as the material varied in the amount of gas that could be extracted. That is to say, every sample was extracted by connecting the desiccator to an oil-pump for a maximum of two minutes.

The identity and amount of the determined gases were checked with standards.

PLANT MATERIAL

A total of eight species was used in these studies, from twenty-one provenances. Six species were conifers or softwood trees of the family Pinaceae, and the rest broadleaved or hardwood trees, giving a considerable taxonomic diversity and reflecting the growing importance of conifers in the world's economy. At the same time a preponderance of ten different provenances of Pinus contorta will be noticed; while this is mainly due to its remarkable tolerance of poor soils including peaty moorlands, it also serves to give a useful comparison of the behaviour of one polymorphic species from different provenances under the experimental conditions.

Table A shows which natural habitats are generally preferred or avoided by these species and Table B gives a list of all species and provenances used, together with details of habitat and location of the parent trees, as far as information is available. All species used were seedlings with the exception of Betula pubescens, and were grown from seeds provided by the Forestry Commission from a world-wide seed collection.

The seeds were sown in plastic tubes filled with a mixture of peat and sand (1:1 by volume) and after four to five months repotted into pots with the same soil mixture. Seedlings obtained from the Forestry Commission in Edinburgh were immediately repotted into the same peat and sand mixture. In the glasshouse all seedlings had 16 hours light per day and after being repotted were watered

Table A. Natural habitats of the species under examination.

Species	Tolerant of:	Intolerant of:
<u>Pinus contorta</u> (Loudon), Lodgepole Pine PINACEAE	climate: high rainfall, sea wind. soil: poor, stony, peat moorland.	drought, shade. limestone.
	Best growth in gravel or swamps.	
<u>Pinus sylvestris</u> (Linnaeus), Scots Pine PINACEAE	climate: drought, frost, wind. soil: clay, loam, chalk, peat.	shade. waterlogging.
	Best growth on dry, sandy soils.	
<u>Picea sitchensis</u> (Bongard) Carrière, Sitka Spruce PINACEAE	climate: high rainfall, soil: moderate dry peat, sand with high water-table.	drought, sea wind. poor, acid soil.
	THRIVES IN PURE SAND.	
<u>Picea mariana</u> (Miller) Britton, Sterns and Poggerberg, Black Spruce PINACEAE	climate: frost, shade, light. soil: acid, stagnant soil/water.	drought, strong winds, shade (as seedlings)
	Best growth on well-drained, moist soils.	
<u>Larix europea</u> (De Candolle), Common Larch PINACEAE	climate: moderate, continental. soil: light loam, rich, shallow peat.	drought, strong winds, frost, shade. deep waterlogged acid peat, highly calcareous.
	Grows best on hillsides watered by springs.	
<u>Larix laricina</u> (Du Roi) K.Koch, Tamarack PINACEAE	climate: wind, frost. soil: wet, dry swamps, silted lake-beds.	drought, shade.
	Grows best on rich and light soils.	
<u>Betula pubescens</u> (Ehrh.) Birch BETULACEAE	climate: wind, frost. soil: well-drained sand, damp poor peat.	shade. ill-drained peat.
	Widely tolerant in varying soils and climates.	
<u>Alnus incana</u> (Moench) Norwegian Grey Alder CORYLACEAE	climate: wind, cold, wet moderate shade. soil: limestone.	acid peat, stagnant soil/water.
	Grows best near running water.	

Table B. List of provenances of species used in flooding tolerance tests with details of habitat and location.

<u>Species</u>	<u>Provenances*</u>	<u>Latitude</u>	<u>Longitude</u>	<u>Elevation</u>	<u>Habitat type</u>
<i>Pinus contorta</i> Ldn.	67(7987)5	55°4' N	131° 49'W	150 m	muskeg/Arctic Island, Alaska
	65(7976)5	45°55'N	121° 40'W	1070 m	? /Peterson Prairie
	69(7987)3	56°50'N	133° 0' W	150 m	muskeg/Petersburg, Alaska
	†65(797)	46°20' -48°20'N	c.124°W	150 m	? /Washington
	65(7124)100	49°40'N	100°W	1000 m	mineral soil/Cypress Hills, Sask.
	68(7116)6	49°10'N	125°50'W	150 m	near Tofofe, Vancouver Is., B.C.
<i>Pinus sylvestris</i> L.	65(7972)1	46°20'N	124°2' W	150 m	coastal dunes/Long Beach, Washington
	65(7114)1	54°30'N	128°35'W	c.305 m	mineral soil/Terrace, B.C.
	65(7951)14	44°35'N	124°5' W	150 m	coastal dunes/Newport, Oregon
	65(7951)5	43°5' N	124°20'W	150 m	coastal dunes/Bandon, Oregon
	†67(4122)11	57°30'N	3°45'W	30 m	sandy loam/Culbin, Moray
	63(41229)	57°30'N	3°10'W	240 m	pedzel/Speymouth, Moray
<i>Picea mariana</i> B.	69(718)	c.45°40'N	c.50°30'W	-	wet organic site/Baeger, Newfoundland
	†67063	45°53'N	77°25'W	500 m	wet organic site/Jack Pine, Spruce, Fir stand.
	5114	48°53'N	80°54'W	300 m	bog site/Black Spruce stand
	5088	48°31'N	80°50'W	300 m	sandy, dry plain/Black Spruce, Jack Pine stand
<i>Picea sitchensis</i> C.	†66(711)1(3)	54°0' N	132°10'W	150 m	Masset, Queen Charlotte Bay, B.C.
<i>Larix europea</i> D.C.	†62(4371)	49°44'N	15°55'E	200-500m	Ylasim, Bohemia, Czechoslovakia
<i>Larix laricina</i>	†67021	45°58'N	77°26'W	500 m	wet organic site/Black Spruce, tamarate stand)
<i>Alnus incana</i> M.	68018	48°50'N	54°44' W	-	cut-over Black Spruce stand
	†66(127)500	56°30'N	4°10'W	200 m	brown earth, Perthshire.

*Numbers refer to the Forestry Commission's provenance register.

†Indicates species used in one provenance only experiments. In further citation the provenance numbers are not given but can be determined by this indication from the above table.

twice with Hoagland's solution (Thomas, Ranson, and Richardson, 1956) and then kept moist with tap-water.

In the glasshouse seedlings selected for experimental flooding were placed in polythene buckets, filled up to the rim with distilled water or Hoagland's solution, and kept at this level with the solution used. The solutions were renewed every month to avoid effects due to changes of pH. In addition to the normal changes due to ion-uptake the pH of the soil solution is likely to be influenced by reduction processes in the acid soil and by changes in the CO₂-pressure during waterlogging of the soil (Ponnamperuma, 1966). During the so-called "recovery-period" the water-table was lowered by emptying the solutions from the buckets. The soil of those plants was then kept moist with tap-water as with all other tree seedlings in the glasshouse.

Some of the 3-4 year old seedlings potted in earth were grown in the experimental garden. From these trees a number were selected for experimental flooding in 3 m² ponds filled with tap-water. The plants were immersed up to the rim of the pots and the water-table was kept constant with tap- and rain-water.

CHAPTER I.

SHOOT AND ROOT MORPHOLOGY IN RELATION TO FLOODING-TOLERANCE.

1. Shoot-Growth

Before one can start to study metabolic changes in tree-seedlings with regard to their flooding-tolerance, it is necessary to classify the species and varieties used by assessing the visual damage caused by experimental flooding of the soil. Further, as it is not the seedling that is of utmost importance in flooding-tolerance, it is necessary to examine the relevance of using tree-seedlings in tests designed to predict the flooding-tolerance of mature trees.

In this present study the growth of some seven month-old coniferous -seedlings was measured in those plants which were flooded with distilled water or Hoagland's solution and also in unflooded control plants. In contrast to earlier work on herbaceous plants (Crawford, 1966) observation of the growth-rate of the seedlings during a three month flooding period is not an adequate test for distinguishing the tolerant plants from the intolerant. All species of trees used in this study have their growth reduced in waterlogged soil (Table 1.1). However, if the growth of the tree-seedlings is further observed, when the water-table is subsequently lowered, then a primary classification of relative flooding-tolerance is possible (Table 1.2). During this so-called "recovery period" some species resume growth to almost the same rate as their unflooded controls, while in others permanent damage appears to have been done to the plants. This classification was checked by means of a second flooding-period (Tables 1.3 & 1.4) and an estimate of relative experimental flooding-tolerance was obtained.

Table 1.1. A comparison of the effect of four month flooding with Hoagland solution and distilled water on the growth in height of tree-seedlings. Growth rates are increments in height in mm/10 days/cm of initial height of tree-seedling. Each value is the mean of 4 measurements.

	Growth rates		
	Control unflooded	Hoagland Solution	Distilled Water
(a) Species			
<i>Pinus contorta</i>	0.86 ± 0.27	0.32 ± 0.10	0.49 ± 0.06
<i>Larix laricina</i>	2.13 ± 0.11	0.81 ± 0.07	0.54 ± 0.06
<i>Picea mariana</i>	1.38 ± 0.10	0.39 ± 0.07	0.34 ± 0.10
<i>P. sitchensis</i>	1.09 ± 0.10	0.50 ± 0.04	0.32 ± 0.06
<i>Pinus sylvestris</i>	1.31 ± 0.29	1.07 ± 0.18	1.04 ± 0.30
<i>Larix europea</i>	1.13 ± 0.07	0.65 ± 0.14	0.47 ± 0.08
(b) Provenances*			
<i>Larix laricina</i> 67021	2.13 ± 0.11	0.81 ± 0.07	0.54 ± 0.06
" " 68018	1.38 ± 0.13	0.72 ± 0.12	0.56 ± 0.05
<i>Picea mariana</i> 69(718)	0.02 ± 0.11	0.02 ± 0.07	0.01 ± 0.01
" " 67063	1.38 ± 0.10	0.39 ± 0.07	0.34 ± 0.10
" " 5114	1.48 ± 0.20	0.35 ± 0.05	0.11 ± 0.02
" " 5088	1.60 ± 0.19	0.51 ± 0.14	0.14 ± 0.06

*Numbers refer to the Forestry Commission's provenance register.

Table 1.2. A comparison of the recovery in growth rate after four months flooding with Hoagland solution and distilled water on the growth in height of tree seedlings. Growth rates are increments in height in mm/10 days/cm of initial height of tree. Each value is the mean of four measurements.

(a) Species	Growth Rates		
	Control unflooded	Hoagland solution	Distilled water
<i>Pinus contorta</i>	0.67 $\pm$ 0.13	0.66 $\pm$ 0.30	0.29 $\pm$ 0.14
<i>Larix laricina</i>	0.52 $\pm$ 0.13	0.71 $\pm$ 0.03	0.07 $\pm$ 0.03
<i>Picea mariana</i>	0.38 $\pm$ 0.05	0.07 $\pm$ 0.03	0.23 $\pm$ 0.08
<i>Picea sitchensis</i>	0.35 $\pm$ 0.07	0.03 $\pm$ 0.03	0.04 $\pm$ 0.04
<i>Pinus sylvestris</i>	0.35 $\pm$ 0.18	0.00	0.00
<i>Larix europea</i>	0.46 $\pm$ 0.06	0.16 $\pm$ 0.06	0.00
(b) Provenances*			
<i>Larix laricina</i> 67021	0.52 $\pm$ 0.13	0.71 $\pm$ 0.03	0.07 $\pm$ 0.03
" " 68018	0.45 $\pm$ 0.04	0.32 $\pm$ 0.12	0.06 $\pm$ 0.02
<i>Picea mariana</i> 69(718)	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01
" " 67063	0.38 $\pm$ 0.05	0.07 $\pm$ 0.03	0.23 $\pm$ 0.08
" " 5114	0.43 $\pm$ 0.03	0.04 $\pm$ 0.03	0.01
" " 5088	0.46 $\pm$ 0.10	0.01 $\pm$ 0.01	0.01

*Numbers refer to the Forestry Commission's provenance register.

Table 1.3. A comparison of the effect of two months reflooding with Hoagland solution and distilled water after a pretreatment of four months flooding and one month lowering of the water-table. Growth rates are increments in height in mm/10 days/cm of initial height of seedling. Each value is the mean of four measurements.

	Growth Rates		
	Control unflooded	Hoagland solution	Distilled water
(a) Species			
<i>Pinus contorta</i>	0.42 ± 0.18	0.24 ± 0.13	0.35 ± 0.06
<i>Larix laricina</i>	0.46 ± 0.08	1.50 ± 0.09	0.11 ± 0.11
<i>Picea mariana</i>	0.18 ± 0.05	0.08 ± 0.03	0.03 ± 0.01
<i>Picea sitchensis</i>	0.22 ± 0.01	0.01 ± 0.01	0.04 ± 0.02
<i>Pinus sylvestris</i>	0.61 ± 0.12	0.20 ± 0.05	0.08 ± 0.03
<i>Larix europea</i>	0.19 ± 0.04	0.04 ± 0.03	0.02 ± 0.02
(b) Provenances*			
<i>Larix laricina</i> 67061	0.46 ± 0.08	1.50 ± 0.09	0.11 ± 0.11
" " 68018	0.36 ± 0.05	0.98 ± 0.15	0.07 ± 0.02
<i>Picea mariana</i> 69(718)	0.0	0.01 ± 0.00	0.0
" " 67063	0.18 ± 0.05	0.08 ± 0.03	0.01 ± 0.01
" " 5144	0.26 ± 0.06	0.04 ± 0.01	0.03 ± 0.01
" " 5088	0.24 ± 0.13	0.01 ± 0.01	0.05 ± 0.01

*Numbers refer to the Forestry Commission's provenance register.

Table 1.4. Number of tree-seedlings out of samples of 4 surviving flooding with either distilled water or Hoagland solution for 18 months in 16-hour days in the glasshouse.

Flooded with Hoagland solution

(a) Species	growing	dormant	dead
<i>Pinus contorta</i>	1	2	1
<i>Larix laricina</i>		4	
<i>Pinus sylvestris</i>			4

(b) Provenances

<i>L. laricina</i> 67021		4	
" " 68018		4	
<i>Picea mariana</i> 69(718)		3	1
" " 5144		1	3
" " 5088		1	1

Flooded with distilled water

(a) Species

<i>P. contorta</i>	4		
<i>Larix laricina</i>	4		
<i>Picea mariana</i>		3	1
<i>P. sitchensis</i>		4	
<i>Pinus sylvestris</i>		4	
<i>Larix europea</i>		3	1

(b) Provenances

<i>L. laricina</i> 67021	4		
" " 68018	1	3	
<i>P. mariana</i> 69(718)		2	2
" " 67063		3	1
" " 5144	1	3	
" " 5088	2	2	

These results are not only of use in the interpretation of the biochemical investigations, reported in the following chapters, but also show a correlation between the natural habitats of those species (Table A). Further, they seem to justify the use of tree-seedlings instead of mature trees for tests in relation to flooding-tolerance.

Finally, a comparison of Tables B, 1.2 - 1.4 shows a correlation between the sites of the parent-trees of different provenances on the one hand, and growth-rates in experimentally-flooded and -unflooded conditions on the other. Thus it seems that those provenances selected for this study have yielded as intended ecological races that vary in their reaction to waterlogged conditions. That is to say, the special adaptations to the particular habitats of those populations is inherited. The two provenances of Larix laricina could well have been distinguished without treatment in waterlogged soil conditions. The less vigorously growing provenances of Newfoundland proved also to be less flood-tolerant. However, different provenances of Picea mariana could not possibly have been distinguished without subjecting them to experimental stress. Only the variations in shoot-growth throughout the experiment showed a correlation with the sites of the parent trees. It is interesting to note that in the flood tolerant species P. mariana the provenances derived from wet organic soils were not tolerant of prolonged submersion periods in distilled water. (Table 1.4) Although a classification was already apparent in relation to flooding tolerance it was nevertheless considered desirable to pursue a rigorous experimental assessment of flooding tolerance.

After two years all plants flooded with distilled water still appeared healthy except two Larix europea specimens and one Picea mariana. This behaviour in distilled water contrasts with those plants flooded with Hoagland's solution, where only 50% of the Pinus contorta seedlings survived well and even Larix laricina had been reduced to a permanently dormant condition, or else severely damaged. This corresponds with Walker's observation (1967) that fertilizer added to waterlogged soil planted with leblolly (Pinus taeda) and slash pine seedlings (Pinus elliottii) increases the plant's mortality. Furthermore, it indicates that a determination of flooding tolerance by means of a growth test of the shoots gives quicker and better results by using Hoagland's solution instead of distilled water. This was to be expected as an application of nitrogen usually stimulates shoot growth more than root growth and the resulting reduction in root growth may contribute to the susceptibility of the species to flooding (cf. Chapter 1.5: Root/Shoot ratio).

As well as these effects of experimental flooding, certain further visible symptoms of flooding damage were observed. The differences in the appearances of the plants during the "recovery-period" are illustrated in photographs 1 - 5.

The main effects of experimental flooding on the appearance of the plants were:

- (a) Flooded soil does not only cause severe damage to the flood-intolerant plants, but a surplus of nutrition can further increase this effect (Photo. 1, 2 & 3).
- (b) For flood-tolerant plants a nutritive solution has the normally expected fertilizer effect (Photo. 4 - 6).

Photograph 1.

Flood-intolerant species

Larix europea (9 months old)

Plant 1.

Flooded in distilled
water for 4 months;
1 month lowering of
water-table

Plant 2.

Unflooded.

Plants 3 and 4.

Flooded in Hoagland
solution for 4 months;
1 month lowering of
water-table.



Photograph 2.

Picea sitchensis (9 months old)

As described for Photograph 1.



Photograph 3.

Flood-intolerant species

Picea mariana (5144) (9 months old)

As described for Photograph 1.



Pinus sylvestris (9 months old)

As describbd for Photograph 1.



Photograph 4.

Flood-tolerant species

Larix laricina 67021 (9 months old)

As described for Photograph 1, except that instead of two plants (3 and 4) for a pretreatment with Hoagland solution, only one (Number 3) was used.



Photograph 5.

Pinus contorta 65(797) (9 months old)



Photograph 6.

Flood-tolerant species

Pinus contorta 65(797) (Two year-old)

Plant 1.

Flooded in distilled
water for 4 months;
1 month's lowering of
water-table, 9 months
reflooded.

Plant 2.

Unflooded.

Plants 3 and 4.

Flooded in Hoagland
solution in parallel
with plant 1.



(c) Variation of flood-tolerance within a provenance was found in Picea mariana, Picea sitchensis and Larix europea; as illustrated by the use of worst and best looking plants for the photographs 1 - 3. When a second flooding period was used variation was also found in Pinus contorta.

(d) Wilting of lower stem leaves starting from the distal position of the leaf is a common symptom of water-logging of the roots even in flood-tolerant species (e.g. Larix laricina and Pinus contorta flooded with Hoagland's solution).

(e) Wilting of the top leaves which is preceded by strong chlorosis when flooded with Hoagland's solution, is however an indication of severe damage by flooding to the roots.

2. Temporary and Permanent Dormancy

During the various growth tests in the glasshouse in which the plants were kept in 16-hour days, as described above (p. 11) it became apparent that experimental flooding influences both temporary and permanent dormancy in the seedlings.* The shoot growth of the seedlings was not continuous as woody plants alternate between flushes or periods of active growth, as well as periods of complete inactivity or dormancy. This same fluctuation between growth and dormancy will also be observed even when the tree seedlings are grown under controlled environmental conditions (Kramer 1957, Lararenne-Allary 1966).

As regards Table 1.5, which gives the duration of initial growth flush of seedlings under different conditions, it is of relevance to note that the onset of dormancy differs in flood-tolerant and intolerant plants. Water-logging with

*For definition see Kramer and Kozlowski (1960)

Table 1.5. Duration of initial growth-flushes in months due to plants being kept in long days and glasshouse conditions. The total duration of the experiment is 28 months from January onwards and the initial age of the seedlings was 9 months. Each value is the mean of four seedlings under observation.

	Unflooded Controls	Flooded in Hoagland Solution	Flooded in Distilled Water
(a) Species			
<i>Pinus contorta</i>	9 ± 3	14 ± 3	16 ± 1
<i>Larix laricina</i>	8 ± 0	14 ± 0	7 ± 0
<i>Picea mariana</i>	9 ± 1	7 ± 2	5 ± 1
<i>Picea sitchensis</i>	8 ± 1	5 ± 1	5 ± 0
<i>Pinus sylvestris</i>	14 ± 0	5 ± 1	4 ± 0
<i>Larix europea</i>	8 ± 0	6 ± 1	5 ± 0
(b) Provenances			
<i>Larix laricina</i> (67021)	8 ± 0	14 ± 0	7 ± 0
<i>Larix laricina</i> (68018)	9 ± 0	13 ± 2	7 ± 0
<i>Picea mariana</i> (69(718))	7 ± 2	13 ± 2	7 ± 0
<i>Picea mariana</i> (67063)	9 ± 1	7 ± 2	5 ± 1
<i>Picea mariana</i> (5144)	8 ± 1	6 ± 0	4 ± 0
<i>Picea mariana</i> (5088)	7 ± 0	4 ± 1	5 ± 0

nutrient solution appears to lengthen the initial growth flush in flood-tolerant seedlings, while an early dormancy seems to be brought about in intolerant species.

Particularly noteworthy is Larix laricina (Photograph 7). Those seedlings whose root-system was submerged in Hoagland's solution clearly revealed three distinctive flushes of growth. The temporary dormancies were characterized by the formation of terminal buds, while the permanent dormancy started with a complete defoliation of the seedlings.

In Larix laricina the trees were flooded with distilled water after being kept in 16-hour days in the glasshouse for a period of eight months. The seedlings lost all their needles and the plants consisted of dormant buds on bare twigs. However, when the trees were flooded with Hoagland's solution this condition was not reached until the 23rd month.

In this context it is also interesting to mention that waterlogging with Hoagland's solution of one and a half year old Picea mariana plants (Provenance 69/718) (Photograph 8) caused an early breaking of bud-dormancy; this would seem to indicate that with those tree seedlings, the mineral nutrition level might have been one of the determining factors in growth extension. However, in Larix europea an early breaking of bud dormancy was observed not only when the seedlings had been flooded with Hoagland's solution but even when the soil was submerged with distilled water (Photograph 9).

Photograph 7.

Effect of experimental flooding on three seedlings of the same age.

Larix laricina 67021

Plant 1.

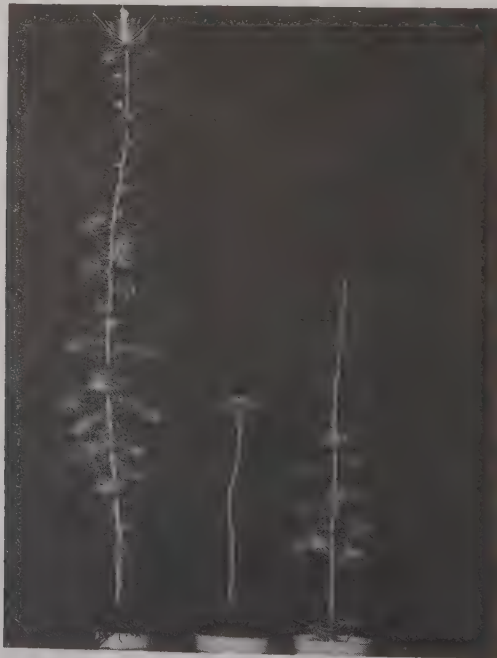
Flooded in Hoagland
solution for 4 months;
1 month's lowering of
water-table, and 21
months reflooded,

Plant 2.

Flooded in
distilled water
in parallel
with plant 1.

Plant 3.

Unflooded.



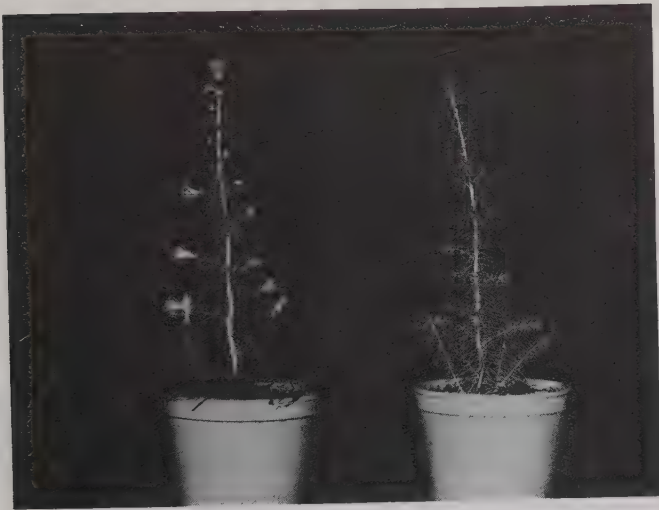
Photograph 8.

Picea mariana 69(718)

Effect of experimental flooding for two months with Hoagland solution.

Plant 1.
Flooded.

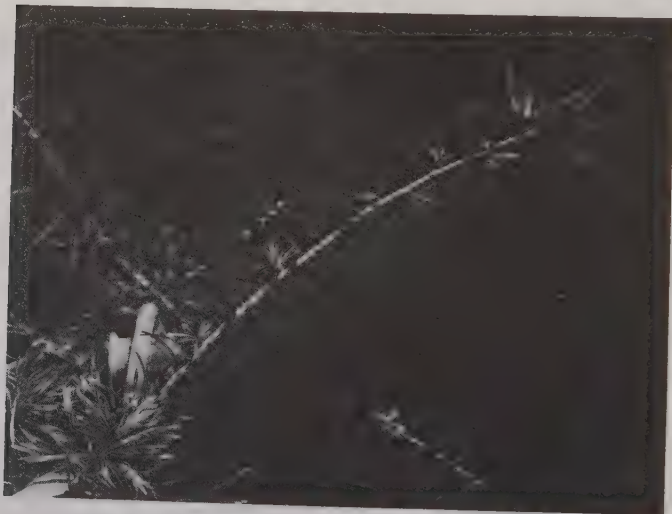
Plant 2.
Unflooded.



Photograph 9.

Larix europea

Early break of bud dormancy when 4-5 year-old tree seedlings have been flooded for 6 weeks with distilled water.



Furthermore the early breaking of bud-dormancy in Larix europea precluded a decay of the whole seedling, while the flood-tolerant Picea mariana (69/718) variety continued to grow. This might indicate that changes in growth regulators are brought about by the flooded conditions. This view seems to be supported by these observations: (1) one to two year old Pinus contorta seedlings continue to grow with short intermittent rest periods for more than 16 months, even when grown in waterlogged soil with distilled water, while at this time the unflooded control seedlings were already severely damaged after being kept in 16-hour days; and (2) in Larix laricina seedlings of the same age the subsequent growth flushes were shortened from fourteen to five and three months, when flooded in Hoagland's solution.

Finally, it may be of interest to mention that only the unflooded Pinus sylvestris seedlings remained undamaged. Control seedlings (unflooded) of Pinus contorta, Picea sitchensis and P. mariana suffered more severely in 16-hour days than seedlings of Larix europea and L. laricina.

3. Adventitious Roots.

Whereas shoot-growth has an inherent periodicity, is is doubtful if this is also the case with root-growth (Hoffmann 1972). Gill (1970) reviewed some anatomical adaptations of flood-tolerant woody species and stressed the importance of adventitious roots at, or below, the water-line.

During the long-term flooding experiments of different

species and varieties in this present study in the glasshouse, the formation of adventitious roots was observed (Photographs 6, 7 and 8). For instance, Alnus incana forms abundantly thick adventitious roots, especially when flooded with distilled or tap water rather than when flooded with nutrient solution. The formation of root-nodules was also adversely affected by waterlogging - again much more noticeably when flooded with Hoagland solution than with distilled water. Thus it would seem that this nutrient solution disturbs somehow the symbiotic balance.

Tables 1.6 and 1.7 provide a summary of the formation of roots on the soil surface in relation to flooding. In general, seedlings which form new roots visible on the soil surface are more successful in withstanding the stress of a surplus water supply; this would seem to be correlated with the pattern of longer shoot-growth periods, in waterlogged conditions. Furthermore, as the formation of new roots on the soil surface is not restricted to conditions where there is waterlogging with distilled water, it may be reasonable to assume that their formation does not correlate with the reduction of nutrition available in submerged soils. This concurs with Jackson's (1958) observation that an increase of nutrient supply does not in fact alleviate the symptoms of flooding (see p.16). Another view is that one main function of the adventitious roots is to produce the growth-promoter, gibberellic acid. Reid and Crozier (1971) noted that the acid accumulates in the shoots of tomato plants after three to four days of flooding, just after the adventitious roots appear. This possible function of the newly-formed roots is

Table 1.6. A comparison of the ability to form roots on the soil surface into the surrounding solution when tree seedlings have been flooded with distilled water or Hoagland solution in the glasshouse. Each observation is based on 4 trees of each species except for the 60 month old seedling when only 2 trees were used per observation.

Species	Provenance	Initial age in months	Observed period in months	Number of plants forming surface roots flooded with:	
				Hoagland solution	distilled water
<i>Alnus incana</i>	66(4127)500	48	24	2	2
<i>Pinus contorta</i>	68(7116)6	6	7	1	0
	69(7987)5	6	7	0	0
	65(7951)H	6	7	0	0
	65(7951)5	6	7	0	0
	65(797)	9	24	1	4
	65(7114)1	60	7	0	2
	65(7124)100	60	7	1	0
	67(7987)5	60	7	0	1
	65(7976)5	60	7	0	0
	65(7972)1	60	7	0	0
<i>Larix laricina</i>	67021	9	24	4	4
	67021	60	7	0	1
	68018	9	24	4	4
<i>Picea mariana</i>	69(718)	9	24	3	3
	67063	9	24	1	0
	5114	9	24	0	4
	5088	9	24	0	1
<i>Picea sitchensis</i>	66(7111)1(3)	9	24	0	4
		18	7	0	0
		30	7	0	0
<i>Pinus sylvestris</i>	67(64122)11	9	24	0	2
		30	7	0	0
	63(41229)	60	7	0	0
<i>Larix europea</i>	62(4371)	9	24	2	1
		35	3	0	0
Total				19	33

Table 1.7. Formation of new roots on the soil surface into the surrounding solution due to experimental flooding in the glasshouse. The data give the duration of experimental flooding in days when the first formation of roots on the soil surface was observed. Each value represents the mean of the positive results out of a sample of four plants.

Species	Provenance	Initial age in months	Observed period in months	Time of first formation of surface roots flooded with: Heagland soln. distilled water	
<i>Alnus incana</i>	66(4127)500	48	24	58 ± 8	48 ± 3
<i>Pinus contorta</i>	68(7116)6	6	7	214	-
	65(797)	9	24	206	169 ± 35
	65(7114)1	60	7	-	140 ± 102
	65(7124)100	60	7	211	-
	67(7987)5	60	7	-	200
<i>Larix laricina</i>	67021	9	24	166 ± 28	166 ± 44
	68018	9	7	-	65
<i>Picea mariana</i>	69(718)	9	24	171 ± 30	177 ± 32
	67063	9	24	45 ± 12	50 ± 30
	5114	9	24	120	-
	5088	9	24	-	192 ± 17
		9	24	-	96
<i>Picea sitchensis</i>	66(7111)1(3)	9	24	-	236 ± 29
<i>Pinus sylvestris</i>	67(64122)11	9	24	-	199 ± 31
<i>Larix europea</i>	62(4371)	9	24	125 ± 5	241

also possible in the tree seedlings which were used (cf. Photograph 7 and I.2 p.17).

Especially interesting is the observation that 60 month-old Pinus contorta seedlings only survive waterlogged conditions with distilled water undamaged for more than eight months if they are able to form roots on the soil surface. On one hand, 9-18 month-old seedlings of all species and varieties used in this study, seem to be able to form roots on the soil surface, but on the other hand, only flood-tolerant species seem to maintain this ability. With older seedlings of flood-intolerant species a formation of roots on the soil surface was never observed.

It is of interest to note in this connection that stem cuttings of previously flooded and unflooded one year old seedlings of Pinus contorta, Larix laricina, Picea sitchensis and P. mariana (Photographs 10, 11 and 12), frequently form adventitious roots in tap water, in contrast to those of Pinus sylvestris and Larix europea which never formed adventitious roots in water culture. Only those cuttings of Pinus contorta and Larix laricina with adventitious roots show a remarkable growth in tap water, in contrast to the behaviour of Picea seedlings. All this suggests that not only are adventitious roots important for plants to withstand the stress of waterlogged soil, but also that some species are more efficient than others. As yet, little is known about the stimulation of the development of adventitious roots; but Kramer has suggested it may be due to a blockage of the downward translocation of carbohydrates and growth-regulating substances (1969). Finally, it may be

Photograph 10.

Pinus contorta 65(797)

Formation of adventitious roots when the stem cutting was kept in tap water for two years.



Photograph 11.

Larix laricina 67021

Formation of adventitious roots when the stem cutting was kept in tap water for two years.



Photograph 12.

Picea mariana 67063

Formation of adventitious roots when the stem cutting was kept in tap water for two years.



pointed out that Gill's description (1970) of the colour of adventitious roots was found to be correct only with Alnus incana seedlings flooded in the open air, and not in any of the other adventitious roots observed. The latter varied from white to dark brown, with red root tips.

4. Shoot Height and Age.

Current theories about flooding tolerance are not confined to the development of adventitious roots. The oxygen-flux from shoot to root is thought to be important, because anaerobic respiration is less efficient and therefore likely to be avoided in plants whenever possible (Crawford, 1972). An oxygen-flux was suggested for seedlings of Salix (sub-species) (Leyton and Rousseau, 1957), and Betula pubescens and B. verucosa (Hurikari, 1954), from experiments in which an indicator was added to the anaerobic medium. In more recent experiments, the more sensitive method of using the oxygen-microelectrode has been applied on flood-tolerant plants (Armstrong, 1967 & 1968; Čirkova, 1968). Greenwood (1967, 1969) has shown too that in vegetable seedlings which are all flood-intolerant species, there is the same phenomenon, viz. an outward diffusion of oxygen into the anaerobic medium surrounding the plants.

As nearly all these studies have been carried out on young plants and seedlings it is possible that the rate of oxygen diffusion may depend more on the age and the height of the plant, rather than on its adaptation to wet land habitats. This possibility does not rule out the force of the observation that seedlings of some species seem to have a higher oxygen-flux from shoot to root than other less flood-tolerant seedlings, as noted by Hurikari (1954). Nor

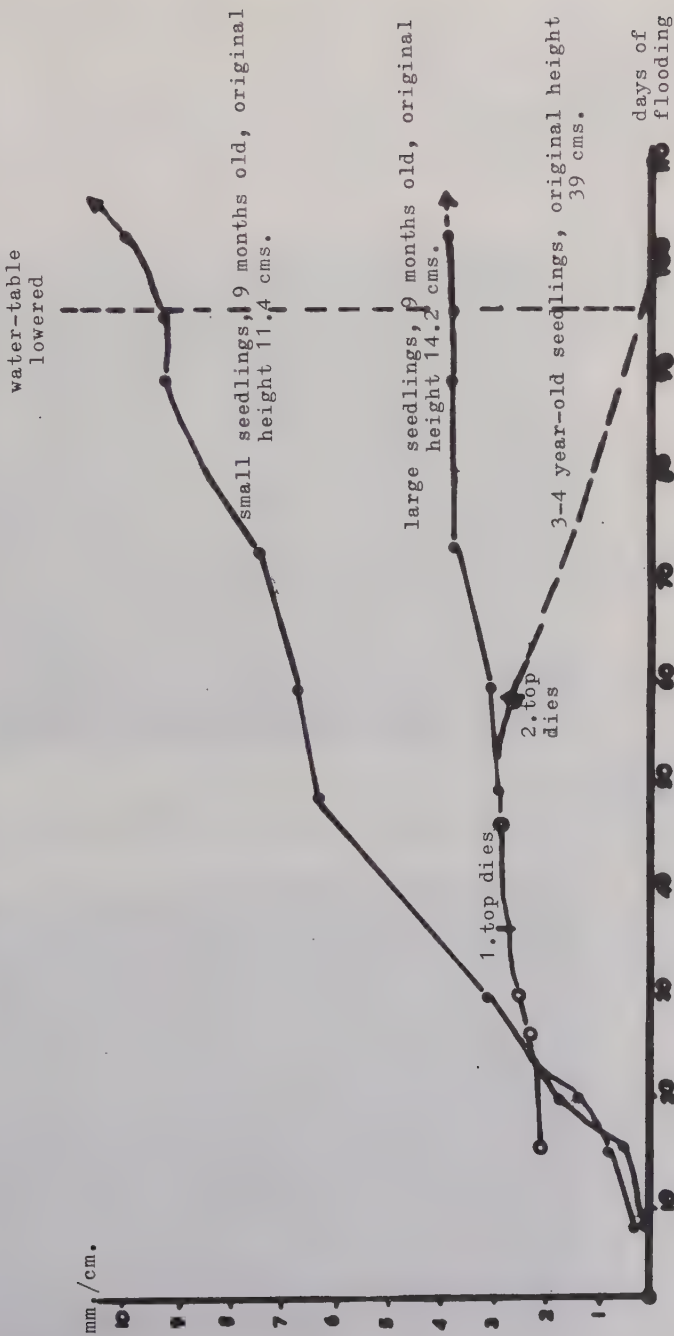
does it exclude the possibility that differences in shoot-height and age may also affect the oxygen-flux to the roots and the resulting flood-tolerance of the species.

Fig. 1.1. shows that taller twelve to sixteen week-old seedlings of Larix europea are more affected in soil waterlogged with Hoagland solution than smaller ones of the same age; and that three to four year old seedlings are even more severely damaged by the same treatment.

Photographs 13 and 14, showing each of the four Picea sitchensis seedlings of different height and age, clearly illustrate that as the age and height of this flood-intolerant species increases, it is correspondingly more easily damaged. Even the effect of nutrient solution accords with this in that the growth of young and small seedlings of this flood-intolerant species is only increased, when the plants are small.

Table 1.8 provides a survey of the shoot-growth of some species of different age and height after they were subjected to experimental flooding. The growth-rates of Picea sitchensis and Pinus sylvestris, as well as the number of their newly-formed side branches (Table 1.9) confirm the earlier statement that plant age and size is inversely related to flooding tolerance. In all species, the larger the tree, the greater the reduction in growth. On one hand, during the first flooding period the formation of new side branches seems to be enhanced in the nine to eighteen month old seedlings of the flood-intolerant species Picea sitchensis. On the other hand, in older tree seedlings of the same species flooding suppresses the formation of new side branches already during this first flooding period.

Fig. 1.1. A comparison of the effect of shoot-height and age on the flooding-tolerance in Hoagland solution of Larix europaea. The increments in height are given in mms per cms of original height. Each value is the mean of two measurements.



Photograph 13.

Picea sitchensis

Damage to seedlings of different age and height during the reflooding period with distilled water (see Table 1.8).



Photograph 14.

As in Photograph 13 but this time the seedlings have been flooded for a prolonged period with Hoagland solution (see Table 1.8)



Table 1.8. A comparison of the effect of waterlogged soil conditions on the shoot growth of tree seedlings of different original height and age. Growth rates are increments in height in mm/10 days/cm of initial height of seedlings. Each value is the mean of at least two measurements. The experiments with 9 month old seedlings were done during spring and summer, the others during autumn and winter in the glasshouse. The values in brackets give approximately the percentage of damaged sheets.

Species	Age of seedling in months	Original height in cms.	Growth Rates Unflooded controls	Flooded with Heagland soln.	Flooded with distilled water.
(1) A first fledging period for 2 months.					
<i>Pinus contorta</i>	9	6.5 ± 0.3	0.86 ± 0.27	0.32 ± 0.10	0.49 ± 0.06
	18	15.7 ± 0.5	0.02 ± 0.01	0.11 ± 0.05	0.14 ± 0.02
<i>Picea sitchensis</i>	9	10.5 ± 0.6	1.09 ± 0.10	0.50 ± 0.04	0.32 ± 0.06
	18	6.3 ± 0.3	0.03 ± 0.03	0.10 ± 0.04	0.02 ± 0.02
	18	23.4 ± 1.4	0.10 ± 0.04	0.02 ± 0.00	0.00
	60	26.1 ± 0.9	0.12 ± 0.03	0.02 ± 0.01	0.08 ± 0.08
<i>Pinus sylvestris</i>	9	40.4 ± 3.1	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01
	60	4.0 ± 0.5	0.31 ± 0.029	1.07 ± 0.18	1.04 ± 0.30
	60	30.3 ± 1.0	0.0	0.0	0.0
(2) A period of lowering the water table for one months.					
<i>Pinus contorta</i>	9	9.9 ± 1.0	0.67 ± 0.13	0.66 ± 0.30	0.29 ± 0.14
	18	16.7 ± 0.6	0.03 ± 0.01	0.24 ± 0.09	0.17 ± 0.06
<i>Picea sitchensis</i>	9	17.3 ± 2.6	0.35 ± 0.07	0.03 ± 0.03	0.04 ± 0.04
	18	6.3 ± 0.3	0.10 ± 0.04	0.11 ± 0.06	0.0
	18	23.9 ± 1.5	0.02 ± 0.01	0.0	0.0
	60	27.1 ± 1.1	0.03 ± 0.02	0.01 ± 0.01	0.0 (25%)
<i>Pinus sylvestris</i>	9	40.4 ± 3.1	0.04 ± 0.02	0.0 (100%)	0.0 (100%)
	60	7.9 ± 0.8	0.35 ± 0.18	0.0	0.0
	60	30.3 ± 1.0	0.0	0.0 (50%)	0.0 (100%)
(3) A refledging period for one month.					
<i>Pinus contorta</i>	9	11.3 ± 1.3	0.42 ± 0.18	0.24 ± 0.13	0.35 ± 0.06
	18	16.9 ± 0.6	0.05 ± 0.02	0.06 ± 0.06	0.04 ± 0.01
<i>Picea sitchensis</i>	9	18.0 ± 2.6	0.22 ± 0.01	0.04 ± 0.01	0.01 ± 0.01
	18	6.4 ± 0.3	0.0	0.0 (50%)	0.0 (25%)
	18	23.9 ± 1.6	0.0	0.0 (25%)	0.0 (50%)
	60	27.1 ± 1.1	0.0	0.0 (100%)	0.0 (50%)
<i>Pinus sylvestris</i>	60	40.6 ± 3.0	0.0	0.0 (100%)	0.0 (100%)
	9	8.1 ± 0.8	0.61 ± 0.12	0.20 ± 0.05	0.04 ± 0.03
	60	30.3 ± 1.0	0.09 ± 0.01	0.0 (100%)	0.0 (100%)

Table 1.9. A comparison of the effect of waterlogged soil conditions on the formation of new side branches on tree seedlings of different original height and age. Each value is the mean of number of side branches in four trees. The experiments with 9 month old seedlings were done during spring and summer, the others during autumn and winter in the glasshouse.

Species	Age of seedling in months	Original height in cms.	Number of sidebranches		
			Unflooded controls	Flooded with Heagland soln.	Flooded with distilled water.

(1) A first flooding period for 2 months.					
Pinus contorta	9	6.5 ± 0.3	0.0	0.0	0.0
	18	15.7 ± 0.5	0.0	0.0	0.0
Picea sitchensis	9	10.5 ± 0.6	1.00 ± 0.41	1.25 ± 0.25	0.0
	18	6.3 ± 0.3	0.0	0.5 ± 0.29	0.5 ± 0.25
	18	23.4 ± 1.4	0.0	0.5 ± 0.29	0.5 ± 0.5
	60	26.1 ± 0.9	1.25 ± 0.48	0.0	0.0
Pinus sylvestris	9	40.4 ± 3.1	0.0	0.0	0.0
	60	4.0 ± 0.5	0.0	0.0	0.0
	9	30.3 ± 1.0	0.0	0.0	0.0
	60	30.3 ± 1.0	0.0	0.0	0.0

(2) A period of lowering the water table for one month.					
Pinus contorta	9	9.9 ± 1.0	0.0	0.0	0.0
	18	16.7 ± 0.6	0.50 ± 0.29	1.00 ± 0.41	0.0
Picea sitchensis	9	17.3 ± 2.6	1.50 ± 0.29	1.25 ± 0.75	0.25 ± 0.25
	18	6.3 ± 0.3	0.50 ± 0.29	1.50 ± 0.29	0.50 ± 0.29
	18	23.9 ± 1.5	1.75 ± 0.48	0.50 ± 0.25	0.25 ± 0.25
	60	27.1 ± 1.1	3.25 ± 0.75	0.0	0.0
Pinus sylvestris	9	40.4 ± 3.1	0.0	0.0	0.0
	60	7.9 ± 0.8	0.0	0.0	0.0
	9	30.3 ± 1.0	0.0	0.0	0.0
	60	30.3 ± 1.0	0.0	0.0	0.0

(3) A reflooding period for one month.					
Pinus contorta	9	11.3 ± 1.3	0.25 ± 0.25	0.0	0.50 ± 0.50
	18	16.9 ± 0.6	0.50 ± 0.29	0.50 ± 0.5	0.0
Picea sitchensis	9	18.0 ± 2.6	0.70 ± 0.48	0.0	0.0
	18	6.4 ± 0.3	0.0	0.0	0.0
	18	23.9 ± 1.6	0.0	0.0	0.0
	60	27.1 ± 1.1	0.0	0.0	0.0
Pinus sylvestris	9	40.6 ± 3.0	0.0	0.0	0.0
	60	8.1 ± 0.8	0.0	0.0	0.0
	9	30.3 ± 1.0	0.0	0.0	0.0
	60	30.3 ± 1.0	0.0	0.0	0.0

These observations would suggest that in mature trees the oxygen supply from the shoot to the root is not sufficient for respiratory needs. Among all the species used, however, Pinus contorta seems to be the most flood-tolerant. Unfortunately, Pinus contorta seedlings of similar provenances and of different ages were not available at one and the same time. Nonetheless, in view of (i) Photograph 15, showing species of different provenances and of different ages and heights, (ii) Photograph 16, showing seedlings of one provenance when flooded and unflooded, and (iii) all the shoot growth data of Pinus contorta seedlings (Tables 1.8 and 1.9), it may be inferred that the flood tolerance of these Pinus contorta seedlings is determined neither by age nor by height. In short, Pinus contorta seedlings seem to be able to adapt their root metabolism positively to anaerobic conditions.

5. Shoot Growth in Glasshouse and Experimental Garden.

(a) Tests with different species.

Growth experiments in a glasshouse are often suspected of not being related to the behaviour of the plants in the field, where many factors other than only a high water-table may be operating. Therefore, some three to four year old seedlings of the flood-tolerant species Alnus incana and the intolerant Larix europea and Pinus sylvestris were flooded under open air conditions and in the glasshouse during a warm and dry summer growing season.

As Table 1.10 shows, it is possible to obtain under both these growing conditions a classification of flood-tolerance. During the experimental period, in the Botanic Garden, the formation of new side branches was suppressed by waterlogged

Photograph 15.

Two Pinus contorta seedlings of different provenances, height and age flooded for a prolonged period.

Plant 1.

Provenance 68(7116)6
(see Table 1.14c)

Plant 2.

Provenance 65(7114)1
(see Table 1.16d)



Photograph 16.

Pinus contorta 65(797)

Plant 1.

Unflooded

Plant 2.

Flooded with Hoagland solution
for 2 months; 1 month lowering
of water-table; 1 month reflooded.



Table 1.10. A comparison of the effect of flooding in the glasshouse (distilled water) and the open air (tap and rain water) on the growth in height of three to four year old seedlings and on the formation of new side branches. Growth rates are increments in height in mm/10 days/cm of initial height of seedling. Each value is the mean of two measurements and of the total number of new sidebranches in two trees.

Species	Duration of experiment	Growth rates		New Side branches	
		Control unflooded	Distilled water	Control unflooded	Distilled water
(a) Glasshouse					
Alnus incana	30 days	0.96 ± 0.09	0.45 ± 0.03	4.5 ± 1.5	1.5 ± 1.5
Larix europea	30 days	1.41 ± 0.19	0.99 ± 0.07	8.5 ± 2.5	0.5 ± 0.5
Pinus sylvestris*	60 days	0.03 ± 0.03	0.0	0.0	0.0
63(4122)11					
Alnus incana	120 days	0.37 ± 0.03	0.36 ± 0.16	5.5 ± 0.5	2.0 ± 1.0
Larix europea	85 days	1.26 ± 0.04	top wilted	22.0 ± 2.0	11.5 ± 1.5
(b) Botanic Garden					
Alnus incana	30 days	0.24 ± 0.08	0.32 ± 0.04	0.5 ± 0.5	1.0 ± 0
Larix europea	30 days	0.97 ± 0.03	0.14 ± 0.01	0.0	0.0
Pinus sylvestris	60 days	0.14 ± 0.00	0.13 ± 0.03	0.0	0.0
63(4122)11					
Alnus incana	120 days	0.31 ± 0.01	0.19 ± 0.04	4.5 ± 1.5	3.0 ± 0
Larix europea	85 days	0.78 ± 0.02	0.13 ± 0.01	0.0	0.0

*Here experimental period: September - November, 1971. All others from June 1970 onwards.

soil conditions in the flood-intolerant species. In addition the main shoot growth was also more reduced in the intolerant species than in Alnus incana. In the glasshouse, in all species, the growth and formation of new side branches was initially reduced, and even Alnus incana seedlings lost approximately 40 per cent of their leaves in waterlogged soil conditions. It might be of interest to note that in the flooded Alnus incana seedlings the wilting of the leaves started around the leaf-veins on the lowest main stem leaves, and then on the proximal branch-borne leaves (Photograph 17), just as if some poison would have slowly been transported from the submerged root system into the stem or some important metabolite from the roots decreased as it was transported upwards. However, as the experiment proceeded, Alnus incana resumed growth, while in the flood-intolerant species the growth reduction continued and severe damage caused through the water-logged soil conditions became obvious. It is likely that the more vigorous growing seedlings under the rather controlled conditions of the glasshouse are less hardened to a sudden environmental change. Moreover, water in ponds, exposed to wind, is less stagnant than solutions in a bucket in a glasshouse.

(b) Provenance test with Pinus contorta.

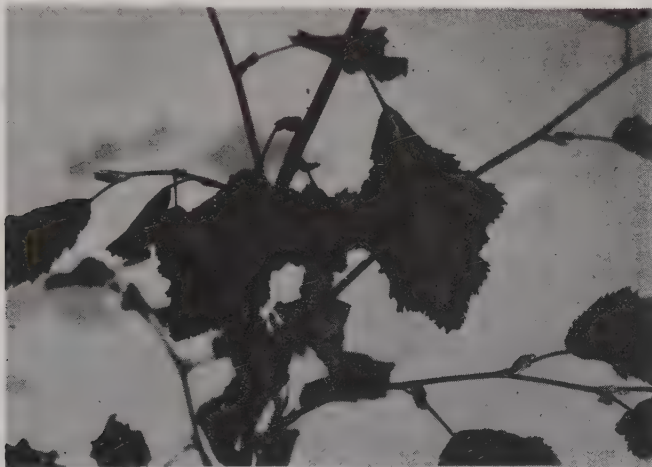
The tests with the tree seedlings of the above species using different provenances in the Botanic Garden and the glasshouse (Table 1.11), as described above, do not show a parallel response to the culture conditions.

The results of the experiments in the glasshouse during the winter months are unexpected. A period of re-flooding with distilled water caused more damage to the seedlings than

Photograph 17.

Alnus incana

Wilting of leaves started around the veins of those leaves proximal to main axis.



Photograph 18.

Alnus incana

Formation of new side branches and leaves after flooding with distilled water for more than one year.

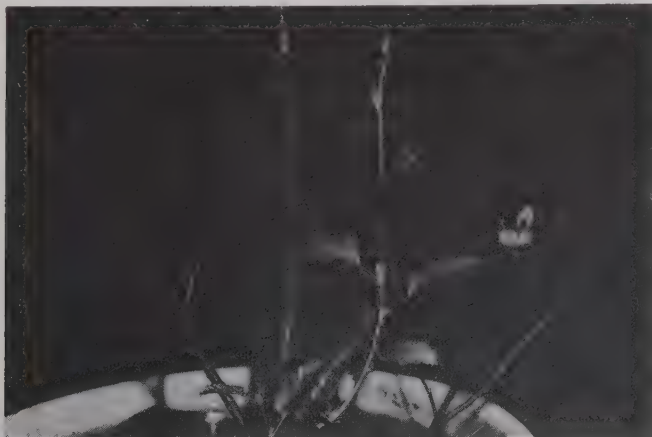


Table 1.11. A comparison of the effect of flooding in the glasshouse and open air on the growth in height of three to five year old *Pinus contorta* seedlings. Growth rates are increments in height in mm/100 days/cm of initial height of tree. Each value is the mean of four measurements, except those flooded in the glasshouse where only two plants were used. The values in brackets give only approximately the percentage of damaged shoots.

(a) Botanic Garden (June 1970 - July 1971)

Provenances	Controls unflooded	Flooded in tap water
65(7976)5	0.27 ± 0.04 (50%)	0.31 ± 0.08
65(7987)5	0.47 ± 0.12	0.28 ± 0.07
65(7124)100	1.05 ± 0.41	0.16 ± 0.05
65(7114)1	0.54 ± 0.06	0.28 ± 0.09 (25%)
65(7972)1	0.65 ± 0.19	0.34 ± 0.11 (50%)

(b) A first flooding period in the glasshouse (September - November, 1971)

Provenances	Unflooded controls	Flooded in Hoagland soln.	Flooded in Distilled water
65(7976)5	0.12 ± 0.12	0.22 ± 0.05	0.0
65(7987)5	0.11 ± 0.04	0.64 ± 0.02	0.32 ± 0.22
65(7124)100	0.24 ± 0.13	0.44 ± 0.31	0.26 ± 0.26
65(7114)1	0.53 ± 0.26	0.62 ± 0.12	0.08 ± 0.02
65(7972)1	0.26 ± 0.15	0.35 ± 0.05	0.13 ± 0.04
65(7976)5 ^z	0.06 ± 0	0.04 ± 0.02	0.04 ± 0.01
65(7972)1 ^z	0.04 ± 0.04	0.0	0.02 ± 0.01

(c) A period of lowering the water table (November - December, 1971)

65(7976)5	0.0	0.0	0.0 (25%)
65(7987)5	1.48 ± 0.28	0.05 ± 0.05	0.20 ± 0.20
65(7124)100	0.28 ± 0.24	0.34 ± 0.34	0.0
65(7114)1	0.19 ± 0.14	0.0	0.0
65(7972)1	0.07 ± 0.07	0.0	0.0
65(7976)5 ^z	0.06 ± 0.03	0.05 ± 0.02	0.04 ± 0.01
65(7972)1 ^z	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02

(d) A reflooding period for five months (December 1971 - May 1972)

65(7976)5	0.01 ± 0.01	0.0 (50%)	0.01 ± 0.01 (90%)
65(7987)5	0.11 ± 0.02	0.01 ± 0.01 (50%)	0.10 ± 0.10 (50%)
65(7124)100	0.09 ± 0.03	0.0 (50%)	0.02 ± 0.02 (90%)
65(7114)1	0.39 ± 0.10	0.13 ± 0.13	0.02 ± 0.02
65(7972)1	0.08 ± 0.06 (25%)	0.0 (29%)	0.04 ± 0.03 (75%)
65(7976)5 ^z	0.02 ± 0.01	0.0 (50%)	0.02 ± 0.02
65(7972)1 ^z	0.03 ± 0.01	0.0 (100%)	0.0 (100%)

^z These plants had been kept in the glasshouse under constant light conditions for two years; the other plants had been in the glasshouse for only one month before the experiment started.

did Hoagland solution. Furthermore, in the open air a clear sequence of flood-tolerance could be obtained, whereas the seedlings in the glasshouse did not reveal such a pattern, rather, the sequence of their flood-tolerance seems to be the inverse of the glasshouse plants especially during the reflooding period. This might be because the cooler period of the year was chosen for the glasshouse experiments, i.e. September to February, but with 16-hour days, and because it is known that trees rarely, if ever, grow continuously (Kramer, 1957). On the other hand, the experiments in the glasshouse may well only indicate that the number of trees flooded was too small, and that a morphological classification of four to five year old seedlings of different provenances is not reliable in such a short time. However, another explanation seems likely and that is, the plants used in some of the glasshouse experiments were newly repotted before the flooding treatment began. It is possible that the amount of time left for the plants to establish themselves in the new environment was too short. This view is substantiated by the fact that in those glasshouse experiments when the plants were not repotted the flooding tolerance of the provenance was found to be in the same order both in the Botanic Garden and glasshouse tests.

Therefore, it seems likely that the classification secured in the Botanic Garden is related to the flooding tolerance of the provenances of Pinus contorta seedlings; also, if the same species of different provenances are kept in the glasshouse for a sufficiently long period after repotting and before the start of the experiment, a similar flood tolerance classification

through experimental flooding in the glasshouse and the open air may be observed.

6. Root-Shoot Ratio.

The results of the effects of flooding on shoot growth of three to four year old Pinus contorta seedlings of different provenances in the glasshouse have not revealed, as already mentioned, a definite flood-tolerance classification. However, during the preparation of plant material for biochemical analysis, it was recognised that the different provenances of Pinus contorta had characteristic root-shoot ratios. A comparison of Tables 1.11a and 1.12 shows a positive correlation between shoot growth in the open and the root/shoot ratio of unflooded P. contorta seedlings. An increase in the natural tendency of a variety to produce roots is accompanied in these experiments by an increased tolerance to flooding.

Regarding the root/shoot ratio, at c. 0.50, the mortality of the seedlings in the Botanic Garden is increased in soil conditions which are waterlogged; at c. 0.75, experimental flooding seems only to decrease the shoot growth; at c. 1.0 the result is low growth rates in both flooded and control plants. Thus, where there is a higher root/shoot ratio, there seems to be a better capability to withstand waterlogged conditions than in other varieties. This is not surprising, since waterlogged soil injures or kills roots, resulting in a restriction of the supply of water and minerals to the plant (Kramer, 1960).

To discover if the possible flooding tolerance classification test for Pinus contorta seedlings is limited

Table 1.12. A comparison of the root/sheet ratio of give provenances of Pinus contorta (three to five year-old seedlings) when grown without flooding. Each value is the mean of 5 measurements.

Provenance	Shoot height in cms	Weight of shoot in gs	Weight of root in gs	Ratio Root wt./shoot wt.
65(7976)5	26.7 ± 2.2	26.4 ± 7.6	24.2 ± 3.3	0.92 ± 0.15
67(7987)5	24.7 ± 1.4	32.5 ± 2.4	35.0 ± 3.5	1.08 ± 0.10
65(7124)100	25.4 ± 1.7	35.3 ± 3.0	26.9 ± 2.1	0.76 ± 0.03
65(7114)1	29.2 ± 2.2	42.7 ± 6.7	21.1 ± 2.6	0.49 ± 0.04
65(7972)1	32.0 ± 2.8	49.6 ± 4.0	23.2 ± 2.9	0.47 ± 0.04
65(7976)5	31.1 ± 2.6	66.6 ± 8.2	73.9 ± 6.8	1.11 ± 0.24
65(7972)1	47.1 ± 2.1	113.6 ± 31.0	44.5 ± 16.1	0.39 ± 0.04

to five year-old plants, the root/shoot ratio was determined (Table 1.13) and compared with shoot/growth (Table 1.14) and formation of new side branches (Table 1.15) during a recovery and reflooding period in seedlings of different provenances, which were only half a year old. In this example also it is seen that where there is a higher root/shoot ratio, one may anticipate a better capability in the seedlings to cope with the stress of a surplus water supply. A comparison of Table B giving details of the habitats of the different provenances with Tables 1.13 and 1.14, shows that these provenances collected from muskeg soils have the highest root/shoot ratio, if kept in the glasshouse. It would seem probable then, that a high root/shoot ratio is a positive selection attribute for waterlogged conditions which is genetically fixed in Pinus contorta.

Table 1.16 A-C shows the root/shoot ratio of a variety of species and provenances of widely varying shoot height and age. It is interesting to note that Picea mariana seedlings reveal the highest root/shoot ratios of all seedlings used in this study.

However, when all these plants of varying age and size are compared it is not possible to obtain a positive relationship between root/shoot ratio and flood-tolerance. Therefore it must be emphasized that this test is only valid if the material is of one species of comparative age and size. Kalela (1950) showed already that there are differences in the rate of root and shoot growth at various ages of trees. In addition, it is to be expected that new repotted tree seedlings have a reduced root weight (Table 1.16 C).

Table 1.13. The root/shoot ratio of some six month old Pinus contorta seedlings in the glasshouse under unflooded conditions. Each value is the mean of eight measurements.

Provenances	Shoot height in cms	Shoot weight in gs	Root weight in gs	Ratio Root/shoot wt.
69(7987)3	3.6 $\pm$ 0.2	1.11 $\pm$ 0.09	1.12 $\pm$ 0.08	1.01 $\pm$ 0.04
68(7116)6	6.4 $\pm$ 0.4	1.34 $\pm$ 0.15	1.04 $\pm$ 0.16	0.77 $\pm$ 0.07
65(7951)14	7.5 $\pm$ 0.6	1.49 $\pm$ 0.15	1.18 $\pm$ 0.15	0.79 $\pm$ 0.09
65(7951)5	8.0 $\pm$ 0.3	2.41 $\pm$ 0.14	1.08 $\pm$ 0.09	0.45 $\pm$ 0.06

Table 1.14. The effect of waterlogged soil conditions on the shoot growth of six month-old Pinus contorta seedlings. Growth rates are increments in height in mm/10 days/cm of initial height. Each value is the mean of four measurements. The values in brackets give the percentage of damaged shoots approximately.

Provenances	Unflooded controls	Flooded with Hoagland solution	Flooded with distilled water
(a) A first flooding period for two months			
69(7987)3	0.09 ± 0.03	0.05 ± 0.02	0.31 ± 0.08
68(7116)6	0.22 ± 0.07	0.0	0.08 ± 0.03
65(7951)14	0.11 ± 0.06	0.02 ± 0.01	0.15 ± 0.03
65 (7951)5	0.09 ± 0.04	0.06 ± 0.04	0.10 ± 0.04
(b) A period of lowering the water table for one month			
69(7987)3	0.03 ± 0.06	0.08 ± 0.06	0.04 ± 0.02
68(7116)6	0.07 ± 0.07	0.03 ± 0.03	0.04 ± 0.04
65(7951)14	0.09 ± 0.06	0.09 ± 0.06	0.17 ± 0.03
65(7951)5	0.07 ± 0.06	0.0	0.29 ± 0.05
(c) A reflooding period of one month			
69(7987)3	0.06 ± 0.03	0.08 ± 0.03	0.05 ± 0.03
68(7116)6	0.06 ± 0.03	0.12 ± 0.09	0.04 ± 0.03
65(7951)14	0.08 ± 0.05	0.02 ± 0.01	0.01 ± 0.01 (25%)
65(7951)5	0.16 ± 0.05	0.03 ± 0.01	0.06 ± 0.03 (25%)

Table 1.15.

The effect of waterlogged soil conditions on the formation of new side branches of six month-old *Pinus contorta* seedlings. Each value is the mean of number of new sidebranches in four tree seedlings.

Provenances	Unflooded controls	Flooded with Hoagland solution	Flooded with distilled water
(a) A first flooding period of two months.			
69(7987)3	0.0	0.0	0.0
68(7116)6	0.0	0.0	0.0
65(7951)14	0.25 ± 0.25	0.0	0.25 ± 0.25
65(7951)5	0.50 ± 0.29	0.0	0.0
(b) A period of lowering the water table for one month			
69(7987)3	0.25 ± 0.25	0.25 ± 0.25	0.75 ± 0.75
68(7116)6	0.25 ± 0.25	0.0	0.0
65(7951)14	1.25 ± 0.48	0.75 ± 0.48	0.50 ± 0.50
65(7951)5	0.50 ± 0.29	0.25 ± 0.25	0.75 ± 0.75
(c) A reflooding period of one month			
69(7987)3	0.0	0.0	0.75 ± 0.75
68(7116)6	0.25 ± 0.25	0.0	0.0
65(7951)14	0.25 ± 0.25	0.25 ± 0.25	0.25 ± 0.25
65(7951)5	0.50 ± 0.29	0.50 ± 0.50	0.25 ± 0.25

Table 1.16. A. The root/shoot ratio of one species of remarkably different shoot height.
 B. The root/shoot ratio of different tree species.
 C. The root/shoot ratio of one species of different age and different pretreatment recently repotted, (b) net.
 Each value is the mean of at least five measurements.

Species	Age in months	Shoot height in cms	Shoot weight in gs	Root weight in gs	Ratio Shoot weight / Root weight
A. <i>Picea sitchensis</i>	18	4.44 ± 0.22	0.65 ± 0.06	0.99 ± 0.08	1.52 ± 0.07
"	18	21.86 ± 0.77	3.28 ± 0.19	2.79 ± 0.20	0.85 ± 0.04
B. <i>Larix laricina</i>	30	59.78 ± 4.94	20.56 ± 1.2	11.92 ± 1.14	0.58 ± 0.05
<i>Picea mariana</i> (69/718)	18	15.04 ± 0.49	1.35 ± 0.07	3.09 ± 0.17	2.29 ± 0.16
<i>Larix europea</i>	60	35.7 ± 4.03	33.89 ± 7.37	27.42 ± 4.86	0.81 ± 0.10
C. <i>Pinus contorta</i>	30	31.82 ± 2.30	19.01 ± 1.98	10.72 ± 1.10	0.56 ± 0.11
"	18	11.92 ± 0.33	1.26 ± 0.06	2.11 ± 0.11	1.68 ± 0.09

Finally, it is interesting to point out that in this study there was no difference between the root/shoot ratio in the flood-tolerant Pinus contorta and the flood-intolerant Picea sitchensis seedlings.

CONCLUSION.

The aim of this study of shoot and root morphology following experimental flooding in the glasshouse was to discover a sequence of flood-tolerance in the species and provenances used. During the so-called recovery period, when the water-table was lowered, the shoot growth proved to be of help in distinguishing between species and provenances which show resistance to flooding (viz. Larix laricina and Picea mariana of different provenances) and those which are severely damaged in waterlogged conditions. Furthermore, with experimental flooding of less tolerant species, the onset of dormancy of the shoot growth was found to be earlier. In the main, however, the root/shoot ratio of Pinus contorta seedlings proved to be conclusive in the determining of the relative flooding tolerance of the different provenances. This sequence of flood-tolerance in tree seedlings (Table 1.17) was obtained from plants grown in the glasshouse under 16-hour days. In addition, experiments in the open air showed that a flood-tolerance classification obtained in a glasshouse is related to the behaviour of different species in the field, viz. natural habitats, origin of seeds and experimental garden flooding treatments. The classification of all the species and provenances used (Table 1.17) is very important for an

ecological interpretation in all the subsequent biochemical experiments (see also Table 1.18).

Not only do all these experiments as described in Chapter 1 reveal a possible flood-tolerance classification, they also show that there are morphological responses to experimental flooding in tree seedlings; (i) shoot growth is initially reduced, an exception being the most flood-tolerant variety of Picea mariana (69/718) when Hoagland solution is used; (ii) the onset of dormancy is affected, viz. whereas intolerant species shorten, more flood-tolerant species lengthen their initial growth flush in a 16-hour day; (iii) the ability to form adventitious roots proved to have increased the flood-tolerance of the species used.

Finally, these experiments have indicated the importance of shoot height and age in seedlings, and how these factors relate to their flooding tolerance. This suggests that an adequate oxygen-flux from the shoot to the root is essentially a characteristic of young seedlings.

Table 1.17. Ranking in decreasing order of flooding-tolerance of the tree seedlings used in this study according to the findings described in Chapter I.

Species	Provenances*	
<i>Pinus contorta</i>	67(7987)5	"flood-tolerant"
<i>Pinus contorta</i>	65(797)	
<i>Pinus contorta</i>	65(7976)5	
<i>Pinus contorta</i>	69(7987)3	
<i>Alnus incana</i>	66(4127)500	
<i>Larix laricina</i>	67061	
<i>Picea mariana</i>	69(718)	
<i>Pinus contorta</i>	65(7124)100	
<i>Pinus contorta</i>	65(7116)6	
<i>Larix laricina</i>	68018	
<i>Pinus contorta</i>	65((7972)1	
<i>Pinus contorta</i>	65(7114)1	
<i>Pinus contorta</i>	65(7951)1H	
<i>Pinus contorta</i>	65(7951)5	
<i>Picea mariana</i>	67063	"Flood-intolerant"
<i>Picea mariana</i>	5144	
<i>Picea mariana</i>	5088	
<i>Picea sitchensis</i>	66(7111)1(3)	
<i>Pinus sylvestris</i>	67(4122)11	
<i>Larix europea</i>	62(4371)	
<i>Pinus sylvestris</i>	63(41229)	

Table 1.18. Summary by ranking of tree seedling performance in relation to flooding as measured by the various morphological tests.

A. 1 - 3 year-old tree seedlings

Species	Appearance of shoot after two years of flooding (see pp. 14 & 15)	Growth rate in recovery period (see Table 1.2)	Growth rate in reflooding period (see Table 1.3)	Initial growth flush (see Table 1.5)	Rank Totals
	Hoagland solution	Hoagland D.W. solution	Hoagland D.W. solution	Hoagland D.W. solution	
Pinus contorta	1	1	1	1	10
Larix laricina	1	2	2	1	13
Picea mariana (67063)	3	1	1	2	31
Picea mariana (5088)	4	3	4	3	36
Picea sitchensis	5	4	6	7	39
Pinus sylvestris	6	5	6	5	41
Larix europea	7	7	3	7	42

B. 4-5 year-old tree seedlings

	Mortality	Growth rate in experimental garden (flooded:unflooded)	Rank totals
Pinus contorta 65(7976)5	1	1	2
Pinus contorta 65(7987)5	2	2	4
Alnus incana 66(4127)500	2	3	5
Pinus contorta 65(7124)100	2	6	8
Pinus contorta 65(7114)1	5	4	9
Pinus contorta 65(7972)1	6	4	10

Conclusion: There is no significant correlation between the ranking of each set of experiments. This might be mainly due to the fact that on one hand tree seedlings never grow continuously and on the other hand the application of additional nutrients changes greatly the effect of waterlogged soil conditions. By means of direct comparison of the growth rates a ranking of the 4-5 year-old Alnus incana seedlings was not possible. Yet the comparison of different varieties of Pinus contorta with Alnus incana seedlings showed that Alnus incana seedlings are less flood-tolerant than some of the Pinus contorta varieties. Further, Alnus incana seedlings suffered severely when they had been flooded with Hoagland solution. Both seem to justify a ranking of Alnus incana seedlings as less flood-tolerant than the Pinus contorta variety 65(797).

CHAPTER II.

Carbon Dioxide Fixation and Evolution from Roots in Relation to Flooding Tolerance.

Any ecologist looking for biochemical differences between plants in relation to their varying fitness for specific habitats is unlikely to meet with much success, if he confines himself to qualitative differences on the metabolic processes. All essential pathways, as for instance glucose metabolism, seem to be the same for all plant species. But if one turns to the quantitative aspects of metabolism, however, a different situation emerges. During the last decade, much knowledge has come to light on the regulation of metabolism. It is therefore to be expected that processes regulating the rates of metabolism will hold the key of the failure or success of plants in different environments (Crawford, 1972).

It is well known that water is not directly injurious to roots, as shown by vigorous growth of roots in well aerated nutrient solutions (Kramer, 1969). However, waterlogging a soil reduces drastically its total gas content displacing air from the non-capillary pore space and influencing strongly the CO_2 and O_2 balance (Williams & Barber 1961; Ponnampetrum et al., 1966). An excess of water in the soil without artificial aeration produces mainly an oxygen deficiency and respiration of roots and soil micro-organisms tend to reduce the oxygen and increase the carbon dioxide concentration.

Further it is well known how important the dark fixation of carbon dioxide is for all organisms. The exclusion of CO_2

from the medium causes bacteria, yeast and fungi either to fail to grow or else to grow slowly (Wood, 1951).

It seemed therefore justifiable in taking $C^{14}O_2$ -fixation and CO_2 evolution of roots as a starting point when investigating the facts which influence the varying flood-tolerance of different species and varieties of tree seedlings.

All data obtained, except those in Chapter II, Part 2, are summarized in Table 2.23 at the end of the conclusion.

1. Unflooded Tree Seedlings

In Chapter I.4 (p.21) it was pointed out that the supply of oxygen from the shoot to the roots seems to be reduced with increasing size and age of the seedling. Furthermore, a dependence on oxygen supply has been established not only for short periods of photosynthesis (Calvin et al., 1951) but also for the CO_2 -fixation of roots (Poel, 1953). It thus seemed desirable to investigate the comparative ability of roots of different species to fix carbon dioxide in air and nitrogen.

Table 2.1. shows the results of such a comparison using roots of one to two year-old tree seedlings, when they have been grown without flooding. In any case, as expected, the $C^{14}O_2$ -fixation was greater when roots were respiring aerobically. However, the interest of the results of the experiments reported in Table 2.1. lies in the comparison of the relative effects of anoxia on CO_2 fixation in different species. The ratio of $C^{14}O_2$ -fixation in roots of tree seedlings in air and in cylinder nitrogen (containing approx. 0.5 per cent oxygen) was found to be related to the extent of damage caused by waterlogged soil in all the species investigated. The

Table 2.1. A comparison of the ability of roots of tree seedlings to fix carbon dioxide under air and nitrogen when they have been grown without flooding.

Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing 50 μC of C^{14} . Time of exposure: 1 hour.

A. A comparison of different species of 1 - 2 year-old tree seedlings.

Species	cpm/g fresh weight/hour		Ratio N ₂ /Air
	Air	N ₂	
<i>Pinus contorta</i>	65,756	64,839	0.99
<i>Larix laricina</i>	14,094	11,588	0.82
<i>Picea mariana</i>	99,360	70,505	0.71
<i>Picea sitchensis</i>	175,604	90,503	0.54
<i>Pinus sylvestris</i>	104,510	50,180	0.48
<i>Larix europea</i>	64,584	25,920	0.40

B. A comparison of varieties of *Picea mariana*

Provenance			
67063	99,360	70,505	0.71
5088	309,312	169,804	0.55

C. A comparison of roots of 4-5 year-old *Alnus incana* seedlings, near the soil surface and those deeper in the soil (at least 17 cm below the soil surface).

Root area			
"upper"	195,840	172,456	0.88
"lower"	275,580	231,734	0.84

comparison of Table 2.1 with Table 1.17 shows this clearly. In flood-tolerant seedlings the level of $C^{14}O_2$ -fixation is not significantly affected by changes in the oxygen supply. But in flood-intolerant species, the level of $C^{14}O_2$ -fixation falls with the reduction of oxygen supply. The relevance of this guide and simple test for flood-tolerance of tree seedlings can be well illustrated by the results obtained with Picea mariana seedlings of two different provenances. Those from a wet organic site (67063) again show a lower dependence on oxygen supply than those from a dry sandy plain (cf. also Table 1.17). In addition, it is interesting to point out that those Picea mariana seedlings from a dry site had both in air and nitrogen the highest CO_2 -fixation rate of the roots of all seedlings used in this study.

Finally, three to four year old seedlings of Alnus incana were selected for this kind of experiment. This was to see if the possible classification test of flood-tolerance in tree seedlings is restricted in its usefulness to one to two year old seedlings. Moreover, the advantage of taller plants is that a good separation between roots near the soil surface and those deeper in the soil is more easily obtained. As is to be expected, the alder can be classified by means of this simple test as a very flood-tolerant species. Worthy of note is that "lower" roots (at least 17 cm below the soil surface) seem to have a greater ability to fix CO_2 ; however, they are more affected by changes in the oxygen supply (Table 2.1).

Furthermore, one has only to look at Photographs 1 - 3 to note the considerable variability of tree seedlings of the same age within one species and of one provenance. The

results summarized in Table 2.2 also reveal, as expected, a rather wide variation in the rate of $C^{14}O_2$ -fixation in roots of Picea sitchensis (66(7111)1(3)). This seems to verify the suggestion that the CO_2 -fixation of roots is a highly sensitive indicator of the vitality of the plants used in these experiments.

Table 2.2 also gives the dates when the experiments were done. Up to the middle of May, the $C^{14}O_2$ -fixation is considerably lower than the level later observed, but the dependence on oxygen supply shows the opposite trend. This might show that Picea sitchensis seedlings are more flood-tolerant during the winter months than during the summer months. Such an increase of flood-tolerance in this species would be in accordance with the observation that in winter, dormant plants can survive many weeks of flooding, with little or no permanent injury; but a single day of flooding during the warm period of the year will seriously damage some species (Kramer, 1969).

As the plants used in the experiments were grown in a glasshouse in 16-hour days, but without air conditioning, the results in Table 2.2 suggest that the $C^{14}O_2$ -fixation rate is strongly influenced by temperature which could be related to the activity of soil-organisms and the higher rates of transpiration resulting from flooding in higher temperatures (Kramer & Jackson, 1954; Erikson, 1965).

In the discussion of further results, it should be borne in mind that in the flood-tolerant species or varieties, the level of $C^{14}O_2$ -fixation is little affected by changes in the oxygen supply. That is to say, the $C^{14}O_2$ -fixation ratio - N_2/Air is not lower than 0.80 when the seedlings have been grown without flooding.

Table 2.2. Seasonal variation in the ability to fix carbon dioxide under air and in nitrogen of Picea sitchensis 66(7111)1(3) roots when the seedlings have been grown without flooding.

Roots: 1.0 g fresh wt. Bating solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing $50\mu\text{C}$ of C^{14} . Time of exposure: 1 hour.

No. of experiment	Date of experiment	cpm/min/g fresh weight air	N_2	Ratio N_2/air
1	22nd March	54,184	32,016	0.59
2	7th May	132,258	97,942	0.74
1-2 mean		93,221 $\pm$ 39,037	64979 $\pm$ 32963	0.67 $\pm$ 0.08
3	16th May	212,856	107,118	0.50
4	5th June	235,296	127,886	0.54
5	11th June	202,884	75,456	0.37
6	26th August	216,144	102,600	0.48
3-6 mean		216,795 $\pm$ 6,781	103,265 $\pm$ 10,781	0.47 $\pm$ 0.04
1-6 mean		175,604 $\pm$ 28261	90,503 $\pm$ 13,568	0.54 $\pm$ 0.05

2. Effect of short period of submerged soil conditions.

In an attempt to elucidate how waterlogged soil actually affects the CO₂-metabolism of roots, some seedlings were selected, and their root-system was flooded with distilled water, or Hoagland solution, as described in Chapter I.

Studies on enzyme-induction in higher plants demonstrated induction in two to three days, in relation to different environments (Hageman & Flesher 1960; Kollöffel, 1969; McManmon & Crawford, 1971). Therefore a possible correlation between the flood tolerance and the C¹⁴O₂-fixation was sought. A preliminary experiment with short term flooding treatments was thought to have confirmed this possibility, and prompted these further experiments into the role of CO₂-fixation and evolution in roots of flood-tolerant species. The results summarized in Table 2.3 show that by means of a short period of flooding, the CO₂-fixation in air is negatively affected in the flood-intolerant species. In the flood-tolerant species (Pinus contorta) the reverse effect is obtained. It thus seems that the CO₂-fixation in air is a better indicator of an initial disturbance of the metabolic balance in flood-intolerant seedlings. However, the comparison of the CO₂-fixation ratio N₂/air in unflooded control plants and those seedlings which have been flooded for four to thirteen days (Table 2.4), shows an unexpected result. By means of waterlogged soil conditions the oxygen dependence of the CO₂-fixation is, in comparison to unflooded seedlings, reduced in flood-intolerant species while in Pinus contorta it is increased. In other words, the results are the inverse to those obtained in the previous classification

Table 2.3. Carbon dioxide fixation by roots flooded for a short period in distilled water (a) under nitrogen (b) under air or O₂.

Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO₃ containing 50 μ C of C¹⁴. Time of exposure: 1 hour.

Species	Days flooded	cpm/min/g fresh wt. unflooded	cpm/min/g fresh wt. flooded	Ratio flooded/unflooded
(a) under nitrogen				
<i>Pinus contorta</i>	4	55,358	59,776	1.08
<i>Picea sitchensis</i>	4	91,241 $\pm$ 7,979	78,351	0.73
" "	10	" "	30,967	0.34
" "	13	" "	49,535	0.54
<i>Larix europea</i>	4	25,272	29,225	1.16

(b) under air (or O₂ in the case of the experiments with *Larix europea* and *Pinus contorta*)

<i>Pinus contorta</i>	4	57,103	69,713	1.22
<i>Picea sitchensis</i>	4	158,649 $\pm$ 25,046	93,190	0.65
" "	10	" "	71,496	0.45
" "	13	" "	73,191	0.46
<i>Larix europea</i>	4	64,272	56,330	0.88

Table 2.4. A comparison of the effect on the ability
 (a) to fix carbon dioxide under air or oxygen, and
 (b) to evolve carbon dioxide under nitrogen, when the root
 system of the one to two year old tree seedlings have been
 flooded with distilled water for a short period and unflooded
 control plants.

Roots: 1.0 g. fresh wt. Bathing solution: phosphate
 buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing
 50 μC of C^{14} . Time of exposure: 1 hour.

Species	Duration of pretreatment in days	Gas phase	Unflooded controls	Flooded
(a) CO_2 fixation ratio N_2/air or oxygen				
<i>Pinus contorta</i>	4	N_2 & O_2	0.96	0.85
<i>Picea sitchensis</i>	4	N_2 & air	0.60 ± 0.07	0.84
" "	10	"	" "	0.43
" "	13	"	" "	0.67
<i>Larix europea</i>	4	N_2 & O_2	0.39	0.51
(b) μl CO_2 evolved				
<i>Picea sitchensis</i>	4		37.9 ± 6.20	49.4
" "	13		" "	50.3

test using only unflooded seedlings (Table 2.1, p.30). This seems to point out that flood-tolerant species follow a different pattern to cope with a short period of flooding from that followed by flood-intolerant species. Thus, it would seem possible that seedlings which have been flooded with distilled water for short period may be used in flood-tolerance tests.

Finally, regarding only the flood-intolerant species Picea sitchensis, the first notable disturbance of $C^{14}O_2$ -fixation (Table 2.4) seems to occur between the fourth and tenth day of waterlogging the soil. This is then followed by a second, but smaller, increase in the ratio N_2 /air. Furthermore, the CO_2 -evolution of the roots is increased through flooding. This could be due to an enhanced anaerobic respiration of the roots because of waterlogged conditions as was proved to be the case in flood-intolerant herbaceous plants (Crawford, 1966). However, the R.Q. during the fixation of $C^{14}O_2$ in air remained less than one even when Picea sitchensis seedlings have been flooded for 13 days.

3. Long Period Flooding.

All one to two year-old seedlings used in these experiments were pre-treated with the same sequence and time intervals of raising and lowering the water-table. On 15th January, all these experiments were begun, but the re-flooding period was of different duration for each species, as recorded in Table 2.5. This table also summarizes the growth in height among the seedlings used during this re-flooding period. Four plants were collected at random for each experiment. A comparison of the growth rates to the sequence of the flood-tolerance classification shows

Table 2.5. A comparison of the growth in height of 1½ - 2 year old tree seedlings in the "reflooding" period with Hoagland solution and distilled water, after a pre-treatment of 4 months flooding and one month lowering of the water table. Growth rates are increments in height in mm/10 days/cm of initial height of the seedlings. Each value is the mean of 2 measurements.

Species	Duration of reflooding in days	Controls unflooded	Growth rates	
			Flooded in Hoagland solution	Flooded in Distilled Water
<i>Pinus contorta</i>	60	0.42	0.43	0.25
<i>Larix laricina</i>	47	0.23	2.09	0.19
<i>Picea mariana</i>	67	0.21	0.05*	0.00
<i>Picea sitchensis</i>	26	0.06	0.09*	
<i>Picea sitchensis</i>	104	0.13		0.02
<i>Pinus sylvestris</i>	99	0.39	0.15	0.18
<i>Larix europea</i>	10	0.41	0.54*	

* only 50% of individuals survived to end of experiment
values not determined.

striking differences (Table 1.3). These are particularly obvious in Larix europea after they have been flooded with Hoagland solution, as well as in the two samples of control plants of Picea sitchensis. It should not be surprising, therefore, if these $C^{14}O_2$ -fixation experiments are not susceptible to easy interpretation as regards the general flood-tolerance typical of each species used.

Even a comparison of the ability of roots to fix carbon dioxide in air and in nitrogen, after the seedlings have been flooded with distilled water or Hoagland solution (Tables 2.6 and 2.7) seems only to give an impression of the momentary growth capacity of the plant selected for the experiment. However, when we recall the results obtained for the total $C^{14}O_2$ -fixation of unflooded seedlings and the results of the growth test (Tables 2.1 and 1.17), it does seem very likely that these seedlings which are most severely affected by changes in the oxygen supply (Ratio N_2 /Air 0.30) would not have recovered again. The long pretreatment may already have caused too serious damage to the seedlings. Tables 2.8 and 2.11 show that lengthy waterlogging enhances, in most species, the $C^{14}O_2$ -fixation of their roots. This could be regarded as a positive adaptation to the unfavourable environment, which reduced considerably the total number of roots, as illustrated in Table 2.12. Yet, the high values of $C^{14}O_2$ -fixation do not seem to have any positive effect on the flood-intolerant species. This may indicate that these flood-intolerant species also produce useless metabolites or toxic substances along with their enhanced CO_2 -fixation.

Table 2.6. A comparison of the ability of roots to fix carbon dioxide under air and nitrogen after they have been flooded with distilled water for a prolonged period. Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing 50 μl of C^{14} . Time of exposure: 1 hour.

Species	cpm/g fresh weight/ min.		Ratio
	air	N_2	
<i>Pinus contorta</i>	144,616	68,016	0.47
<i>Larix laricina</i>	61,776	33,943	0.55
<i>Picea mariana</i>	362,765	91,625	0.25
<i>Picea sitchensis</i>	312,668	96,268	0.31
<i>Pinus sylvestris</i>	660,519	398,896	0.60

Table 2.7. A comparison of the ability of roots to fix carbon dioxide under air and nitrogen after they have been flooded with Hoagland solution for a prolonged period.

Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing 50 μC of C^{14} . Time of exposure: 1 hour.

Species	cpm/g fresh weight/min.		Ratio
	air	N_2	
<i>Pinus contorta</i>	289,044	123,752	0.43
<i>Larix laricina</i>	85,473	59,100	0.69
<i>Picea mariana</i>	171,820*	86,180*	0.50
<i>Picea sitchensis</i>	66,143*	19,614*	0.29
<i>Pinus sylvestris</i>	424,600	102,104	0.24
<i>Larix europea</i>	675,486*	289,204*	0.43

*only 50% of individuals survived to end of experiment.

Table 2.8. $C^{14}O_2$ fixation under nitrogen by roots of tree seedlings when they have been flooded for a prolonged period with Hoagland solution and the unflooded controls. Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M $NaHCO_3$ containing 50 μC of C^{14} . Time of exposure: 1 hour.

Species	Controls unflooded	cpm/min./g fresh wt. flooded	Ratio flooded/unflooded
<i>Pinus contorta</i>	64,839	124,752	1.92
<i>Larix laricina</i>	11,588	59,100	5.1
<i>Picea mariana</i>	70,505	86,180*	1.22
<i>Picea sitchensis</i>	74,456	19,614*	0.26
<i>Pinus sylvestris</i>	50,180	120,124	2.04
<i>Larix europea</i>	25,920	289,200*	11.16

*only 50% individuals survived to end of experiment.

Table 2.9. $C^{14}O_2$ fixation under air by roots of tree seedlings when they have been flooded for a prolonged period (compare Table 2.5) with Hoagland solution, and the unflooded controls.

Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M $NaHCO_3$ containing 50 μC of C^{14} . Time of exposure: 1 hour.

Species	cpm/g fresh weight/min.		Ratio Flooded/Unflooded
	Controls unflooded	Flooded	
<i>Pinus contorta</i>	65,756	289,044	4.4
<i>Larix laricina</i>	14,094	85,473	6.07
<i>Picea mariana</i>	99,360	171,820*	1.73
<i>Picea sitchensis</i>	202,884	66,143*	0.33
<i>Pinus sylvestris</i>	104,510	424,600	4.06
<i>Larix europea</i>	64,584	675,486*	10.46

*only 50% of individuals survived to end of experiment.

Table 2.10. $C^{14}O_2$ fixation under nitrogen by roots of tree seedlings when they have been flooded for a prolonged period with distilled water, and unflooded controls. Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M $NaHCO_3$ containing 50μ of C^{14} . Time of exposure: 1 hour.

Species	cpm/g fresh weight/min.		Ratio Flooded/Unflooded
	Controls unflooded	Flooded	
<i>Pinus contorta</i>	64,839	68,016	1.05
<i>Larix laricina</i>	11,588	33,943	2.93
<i>Picea mariana</i>	70,505	91,625	1.3
<i>Picea sitchensis</i>	102,600	96,268	0.94
<i>Pinus sylvestris</i>	50,180	398,896	7.95

Table 2.11. $C^{14}O_2$ fixation under air by roots of tree seedlings when they have been flooded for a prolonged period with distilled water and unflooded controls. Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M $NaHCO_3$ containing 50 μC of C^{14} . Time of exposure: 1 hour.

Species	cpm/g fresh weight/min.		Ratio Flooded/Unflooded
	Controls unflooded	Flooded	
<i>Pinus contorta</i>	65,756	144,616	2.2
<i>Larix laricina</i>	14,094	61,776	4.38
<i>Picea mariana</i>	99,360	362,765	3.65
<i>Picea sitchensis</i>	216,710	312,668	1.45
<i>Pinus sylvestris</i>	104,510	660,519	6.32

Table 2.12. The reduction of root weight of some one to one and half year old seedlings when they have been flooded for 4 months, the water table lowered for one month and then the seedlings reflooded for different periods, as compared with unflooded control seedlings. Each value is the mean of two measurements.

Species	Duration of reflooding in days	Root weight in g		
		Controls unflooded	Flooded in Hoagland solution	Flooded in distilled water
<i>Pinus contorta</i>	60	6.04	2.01	2.06
<i>Larix laricina</i>	47	7.28	2.70	2.25
<i>Picea mariana</i>	67	7.10	0.40*	0.29
<i>Picea sitchensis</i>	26	4.98	0.43*	/
<i>Picea sitchensis</i>	104	10.68	/	0.36
<i>Pinus sylvestris</i>	99	5.87	0.98	0.76
<i>Larix europea</i>	104	8.06	0.63*	/

*only 50% individuals survived to end of experiment.

/ values not determined.

For instance, a high rate of CO_2 -fixation, combined with a similar increase in the rate of CO_2 -evolution, would not result in actual net gain to the roots. In fact, as Tables 2.13 and 2.14 show, the CO_2 -evolution of the roots increases in all species, with the exception of the flood-tolerant Pinus contorta, when the seedlings have been flooded for a prolonged period. Yet when one juxtaposes these results and those of C^{14}O_2 -fixation in nitrogen, as in Tables 2.15 and 2.16, it is seen that in most cases the CO_2 -evolution increases proportionately less, than the fixation. And so, in the flood-intolerant species Pinus sylvestris and Larix europea at least, one can postulate an accumulation of toxic metabolites in the roots, in contrast to the flood-tolerant species Larix laricina and Pinus contorta.

Spurr's mention (1964) of the black spruce (Picea mariana) being the only tree which can grow in stagnant oxygen-scarce water and the central position of this species (Table 1.17) between flood-tolerant and intolerant species, justifies a special interest in Picea mariana seedlings of different provenances. In Chapter I (p.13) it was noted that seedlings with the provenance number 5088 from a dry sandy plain suffered less from waterlogging with distilled water than those with the provenance number 67063, from a wet organic site. However, the comparison of the data of both varieties obtained during the C^{14}O_2 -fixation experiments (Table 2.17) reveals that the seedlings from the dry site (5088) have not only a lower C^{14}O_2 -fixation rate in their roots, but also a more reduced net gain of carbon after having been flooded. Yet, the C^{14}O_2 -fixation of the roots of those plants from the dry site (5088) is much more oxygen

Table 2.13. Carbon dioxide evolution under N₂ in μ l/g fresh weight/hour of roots of tree seedlings that have been flooded in distilled water for a prolonged period compared with unflooded controls.

Species	cpm/g fresh weight/min		Ratio Flooded/unflooded
	Unflooded controls	Flooded	
<i>Pinus contorta</i>	82.5	74.4	0.90
<i>Larix laricina</i>	53.7	78.7	1.47
<i>Picea mariana</i>	62.7	172.2	2.75
<i>Picea sitchensis</i>	48.8	105.8	2.17
<i>Pinus sylvestris</i>	81.9	162.4	1.98

Table 2.14. Carbon dioxide evolution under N₂ in μ l/g fresh weight/hour of roots of tree seedlings that have been flooded in Hoagland solution for a prolonged period, compared with unflooded controls.

Species	cpm/g fresh weight/min		Ratio Flooded/unflooded
	Unflooded controls	Flooded	
<i>Pinus contorta</i>	82.5	80.6	0.98
<i>Larix laricina</i>	53.7	92.3	1.72
<i>Picea mariana</i>	62.7	287.8*	4.59
<i>Picea sitchensis</i>	40.1	53.7*	1.34
<i>Pinus sylvestris</i>	81.9	88.0	1.07
<i>Larix europea</i>	20.9	30.1*	1.44

*only 50% individuals survived to end of experiment.

Table 2.15. Carbon dioxide fixed/100 μ l of CO_2 evolved/
1 g fresh weight/hour of roots of tree seedlings under
 N_2 that have been flooded for a prolonged period in
distilled water, as compared with unflooded controls.
Roots: 1.0 g fresh wt. Bathing solution: phosphate
buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing
50 μ of C^{14} . Time of exposure: 1 hour,

Species	Controls unflooded	Flooded	Ratio Flooded/unflooded
<i>Pinus contorta</i>	78,593	91,419	1.16
<i>Larix laricina</i>	21,579	43,130	2.0
<i>Picea mariana</i>	112,448	53,209	0.47
<i>Picea sitchensis</i>	90,991	333,603	3.67
<i>Pinus sylvestris</i>	61,270	245,626	4.01

Table 2.16. Carbon dioxide fixed/100 μ l of CO_2 evolved/
1 g fresh weight/hour of roots of tree seedlings under
 N_2 that have been flooded for a prolonged period in
Hoagland solution, as compared with unflooded controls.
Roots: 1.0 g fresh wt. Bathing solution: phosphate
buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO_3 containing
50 μC of C^{14} . Time of exposure: 1 hour.

Species	Controls unflooded	Flooded	Ratio Flooded/unflooded
<i>Pinus contorta</i>	78,593	154,776	1.97
<i>Larix laricina</i>	21,579	64,030	2.97
<i>Picea mariana</i>	112,448	29,944*	0.27
<i>Picea sitchensis</i>	185,676	32,782*	0.18
<i>Pinus sylvestris</i>	61,270	116,050	1.89
<i>Larix europea</i>	124,019	960,810*	7.75

*only 50% of individuals survived to end of experiment.

Table 2.17. $C^{14}O_2$ fixation and CO_2 evolution of Picea mariana from different provenances when the seedlings have been flooded with distilled water, as compared with unflooded control plants.

Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M $NaHCO_3$ containing 50 μC of C^{14} . Time of exposure: 1 hour.

Provenances	Unflooded Control Plants	Flooded in Distilled Water	Ratio
(a) $C^{14}O_2$ fixation under N_2 (cpm/g fresh weight/min.)			
67063	70,505	91,625	1.30
5088	169,804	101,768	0.60
(b) $C^{14}O_2$ fixation under air (cpm/g fresh weight/min.)			
67063	99,360	362,765	3.65
5088	309,312	131,800	0.43
(c) $C^{14}O_2$ fixed/100 μl CO_2 evolved under N_2 (cpm/g fresh Wt./min.)			
67063	112,448	53,209	0.47
5088	333,603	79,506	0.24
(d) Comparison $C^{14}O_2$ fixation under N_2 and air (Ratio N_2 /air)			
67063	0.71	0.25	
5088	0.55	0.77	

37.

independent than that of the plants from the wet site (67063). This seems to be the important fact in that instead of comparing two provenances as with P. mariana, we can compare the behaviour of $C^{14}O_2$ -fixation in two different flooding conditions (a) distilled water of which the species is tolerant, and (b) Hoagland solution of which it is intolerant. The same pattern could be found in the species Pinus sylvestris. Seedlings having been flooded with Hoagland solution show a higher enhanced $C^{14}O_2$ -fixation than those with the pre-treatment of distilled water. Yet, only seedlings which have been flooded with distilled water show the remarkable independence on oxygen supply (Ratio $N_2/air = 0.60$) of their $C^{14}O_2$ -fixation in the roots.

In Chapter I it was found that waterlogging with Hoagland solution yields quicker results for a possible flood-tolerance classification than distilled water, hence the data of Tables 2.15 and 2.16 were compared (Table 2.18). The outcome is a clear distinction between two groups of flood-tolerant and intolerant species. The net gain of CO_2 in the roots of flood-tolerant species is higher after a prolonged treatment with Hoagland solution than with distilled water. The converse was found with flood-intolerant species. This underlines, then, the close association between CO_2 -fixation in roots and the ability to withstand waterlogged conditions, as well as the importance of nutritions in relation to the flooding-tolerance of the species.

Table 2.18. A comparison of the ability of roots to fix carbon dioxide/100 μ l carbon dioxide evolved/1 g fresh weight/hour under nitrogen, when the seedlings have been flooded with (a) distilled water, or (b) Hoagland solution for a prolonged period.

Species	Ratio Distilled water pretreatment(a)	Ratio Hoagland soln. pretreatment(b)	Ratio b/a
<i>Pinus contorta</i>	1.16	1.97	1.70
<i>Larix laricina</i>	2.0	2.97	1.49
<i>Picea mariana</i>	0.47	0.27	0.58
<i>Picea sitchensis</i>	3.67	0.18	0.05
<i>Pinus sylvestris</i>	4.01	1.89	0.47

A further example of the dependence of CO_2 -fixation in roots on flooding tolerance was found by comparing seedlings of the same species, some of which were quite healthy and some of which were severely damaged after being pretreated in the same way (Table 2.19). The C^{14}O_2 -fixation decreased in seedlings that were obviously damaged. Where there is in damaged tree seedlings a low C^{14}O_2 -fixation, any increase of the CO_2 -evolution, or in other words, any further decrease in carbon net gain, seems to prelude the total decay of the flood-intolerant seedlings.

The dependence of the C^{14}O_2 -fixation on oxygen supply has been discussed above. However, it is important to note that the data obtained with obviously damaged plants do not fit into the mentioned description. In roots of severely damaged plants the C^{14}O_2 -fixation seems not only to be generally suppressed but there seems to occur also a distinctive inhibition under air. That is to say that the ratio of C^{14}O_2 -fixation N_2/air increases again.

These experiments on long-term flooding were not constricted to one to two year old tree seedlings, but included also three to four year old Alnus incana seedlings. On one hand, Alnus incana is generally known to be a markedly flood-tolerant tree species in a poor soil with moving water, but on the other hand, in this study, a pre-treatment with stagnant water or Hoagland solution was applied. Table 2.20 shows that the pre-treatment with Hoagland solution lasted only 25 days while the pre-treatment with distilled water was prolonged by lowering the water-table for 20 days and reflooding for 79 days. This variation in the pre-treatment was thought necessary as flooding with Hoagland solution damaged Alnus incana seedlings severely,

Table 2.19. Comparison of the CO₂ metabolism of roots of individuals that have survived prolonged flooding although severely damaged, of the two flood-intolerant species Larix europea and Picea sitchensis.

Species	Undamaged plants	Damaged plants	Ratio Damaged/Undamaged plants
(a) C ¹⁴ O ₂ fixation under nitrogen (cpm/g fresh wt/min.)			
Larix europea	289,200	200,047	0.69
Picea sitchensis	19,614	17,604	0.90
(b) C ¹⁴ O ₂ fixation under air (cpm/g fresh wt/min.)			
Larix europea	675,486	421,920	0.63
Picea sitchensis	66,143	19,981	0.30
(c) C ¹⁴ O ₂ fixation/100 µl CO ₂ evolved under N ₂ (cpm/g fresh wt/min)			
Larix europea	960,810	473,905	0.49
Picea sitchensis	83,820	11,135	0.13
(d) Comparison of C ¹⁴ O ₂ fixation under nitrogen and air (Ratio N ₂ /air)			
Larix europea	0.43	0.47	
Picea sitchensis	0.30	0.88	

Table 2.20. A comparison of the effect on growth in shoot height, root weight and number of root nodules greater than 0.8 cm diameter of Alnus incana seedlings, when they have been flooded with Hoagland's solution for 25 days, and with distilled water for 79 days. The growth rates are increments in height in mm/original height in cm/10 days. The root weight is given in g. Lower roots are at a depth of more than 17 cm from the soil surface. Each value is the mean of two measurements.

	Controls unflooded	Flooded in Hoagland solution	Flooded in distilled water
Growth rates of the shoot	0.69	0.09	0.54
Wt. of "upper" roots	660	517	830
Wt. of "lower" roots	385	394	85
No. of root nodules >0.8 cm diameter	20	16	14

even in such a short period as 25 days. Table 2.21 records the data of $C^{14}O_2$ -fixation and CO_2 -evolution of these seedlings. The roots show an increasing dependence on oxygen supply especially if flooded with Hoagland solution. However, the higher values of lower roots compared with those of the upper roots seem to indicate only a higher inhibited $C^{14}O_2$ -fixation under air than under nitrogen (viz. Table 2.19) of an already generally suppressed $C^{14}O_2$ -fixation. The fact that different time intervals were chosen as pre-treatments seems to explain the higher $C^{14}O_2$ -fixation of the "upper" roots of the seedlings when flooded with Hoagland solution. However, regarding the highly disturbed $C^{14}O_2$ -fixation of the "lower" roots of the seedlings when flooded with Hoagland solution, the damaging effect of this nutrient solution is noteworthy. Further there is a drastic decrease in CO_2 -evolution from these "lower" roots when flooded with Hoagland solution. Yet, Alnus incana can survive a long term treatment with stagnant high water-table in the glasshouse, especially when flooded with distilled water, as shown in Photograph 18. New side-branches and leaves were formed after flooding for more than a year. Therefore, the net gain of carbon of the roots was compared with seedlings when they were flooded or unflooded (Table 2.22). Only the upper roots, when flooded with distilled water seem to be unaffected through the changed environment. In this context it is important to remember that in distilled water, in contrast to waterlogging with Hoagland solution, an enhanced formation of "upper", or on the soil surface growing, roots had been observed (cf. Table

Table 2.21. A comparison of the CO₂ fixation and evolution of "upper" (to 17 cm below the soil surface) and "lower" roots of Alnus incana, when three to four year old seedlings have been flooded with distilled water for 79 days or Hoagland's solution for 25 days.
Roots: 1.0 g fresh wt. Bathing solution: phosphate buffer, 3.0 ml pH 5.6, 0.5 ml of 0.31 M NaHCO₃ containing 50 µC of C¹⁴. Time of exposure: 1 hour.

Root area	Flooded in distilled water	Flooded in Hoagland solution	Ratio Hoagland soln./distilled water
(a) C ¹⁴ O ₂ fixation under Nitrogen (cpm/g fresh wt./min.)			
"upper" roots	75,016	115,792	1.54
"lower" roots	71,506	24,015	0.34
(b) C ¹⁴ O ₂ fixation under air (cpm/g fresh wt./min.)			
"upper" roots	114,480	210,516	1.84
"lower" roots	90,004	39,028	0.43
(c) CO ₂ evolution under nitrogen (µl)			
"upper" roots	74.4	100.0	1.34
"lower" roots	75.4	5.6	0.07
(d) comparison of C ¹⁴ O ₂ fixation under nitrogen and air (Ratio N ₂ /air)			
"upper" roots	0.65	0.55	
"lower" roots	0.79	0.62	

Table 2.22. A comparison of the $C^{14}O_2$ fixed/100 μl CO_2 evolved under nitrogen when three to four year-old Alnus incana seedlings have been flooded (a) with Hoagland solution for 25 days only, or (b) distilled water for 79 days, and unflooded controls.

Root area	Unflooded controls	Flooded in a) Hoagland soln. for 25 days	Ratio Flooded/ Unflooded
"upper" roots	99,109	153,570	1.55
"lower" roots	379,892	101,071	0.27
b) Distilled water for 79 days			
"upper" roots	99,109	100,828	1.02
"lower" roots	379,892	71,506	0.19

2.20 and Chapter 1, p.18). This might help those three to four year-old seedlings of Alnus incana to survive well, in the long run, in such a soil condition as a stagnant water-table. In other words, the three to four year old seedlings of the flood-tolerant species Alnus incana seem not to be able to adapt the CO₂ metabolism of their root system to continuous severe anaerobis.

In the flood-intolerant species the RQ in air of the roots increased higher than one, after the seedlings had been flooded for a long period, but even after prolonged flooding, the R.Q. remained smaller than one in the flood-tolerant species Pinus contorta, Larix laricina and Alnus incana.

CONCLUSION

1. A comparison of the ability of roots from unflooded plants to fix $C^{14}O_2$ under nitrogen and air gives an exact parallel of their ability to withstand flooding as discussed in Chapter I, i.e. the fixation rate is less affected by anaerobic conditions in the flood-tolerant species.
2. The effect of 4 days of flooding on the $C^{14}O_2$ -fixation of the roots under oxygen appeared to give a classification that also matches the flooding-tolerance of the trees as determined by growth experiments in Chapter I.
3. An enhanced $C^{14}O_2$ -fixation and net gain of carbon to the roots after a long flooding treatment is not related to flooding tolerance.
4. Flooding for a long period with Hoagland solution increases the net gain of carbon by roots in flood-tolerant species much more than flooding with distilled water. This nutritional response therefore accentuates the differences in the net gain on carbon between flood-tolerant and flood-intolerant species.
5. The CO_2 -exchange in Alnus incana roots is irregular after the plants were flooded with Hoagland solution. However, when the plants were flooded with distilled water the $C^{14}O_2$ -fixation of the upper roots remained unchanged. Waterlogging with distilled water is always followed by an enhanced formation of adventitious roots on the soil surface (Chapter I, p.18). This might enable these seedlings to grow well, even if their CO_2 metabolism seems to be not well adapted to continuously anaerobic soil conditions.

6. The dependence of the $C^{14}O_2$ -fixation of roots on oxygen supply seems to be important when seedlings have been flooded for a long period. Seedlings which are most severely affected by changes in the oxygen supply (Ratio N_2/Air 0.30) belong to the flood-intolerant group. When seedlings of one species from different provenances are compared, those that survive best have a greater independence on their $C^{14}O_2$ -fixation on oxygen supply.

7. However, the comparison of the dependence on the oxygen supply of the $C^{14}O_2$ -fixation of the roots after a long pretreatment does not reveal a sequence of the flooding-tolerance in all the species used in this study. A comparison of plants which were healthy and some which were severely damaged showed a clear case of independence of oxygen supply of the $C^{14}O_2$ -fixation in the roots of the damaged plants.

8. Only in flood-intolerant species, after the seedlings have been flooded for a long period, is the R.Q. of the roots increased above one.

Table 2.23. Summary of data obtained with seedlings when they have been flooded for a prolonged period together with unflooded control plants. Each value is the result of one experiment using two plants collected at random.

A. Different species of 1 - 2 year-old tree seedlings.

Species	Unflooded control plants				Flooded with distilled water				Flooded with Hoagland solution			
	C14O2-fixation		CO2 evolution		C14O2-fixation		CO2 evolution		C14O2-fixation		CO2 evolution	
	cpm/g fresh	weight/hour	pl/g fresh	weight/hour	cpm/g fresh	weight/hour	pl/g fresh	weight/hour	cpm/g fresh	weight/hour	pl/g fresh	weight/hour
	under	under	under	under	under	under	under	under	under	under	under	under
	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
Pinus contorta	65756	64839	82.5	144616	68016	74.4	289044	124752	80.6			
Larix laricina	14094	11588	53.7	61776	33943	78.7	85473	59100	92.3			
Picea mariana	99360	70505	62.7	362765	91625	172.2	171820	86180	287.8			
Picea sitchensis	175604	90503	48.8	312668	96268	105.8	66143	19614	53.7			
Pinus sylvestris	104510	50180	81.9	660519	398896	162.4	424600	102104	88.0			
Larix europaea	64584	25920	20.9				675486	289204	30.1			

B. Varieties of *Picea mariana* (see Table 2.17).

Provenance	99360	70505	62.7	362765	91625	172.2
67063	309312	169804	50.9	131800	101768	128.0
5088						

C. Roots near the soil surface and those deeper in the soil (at least 17 cms below the soil surface) of 4-5 year-old *Alnus incana* seedlings (see Table 2.21 and 2.22).

Root area	195840	172456	174.0	114480	75016	74.4	210516	115792	100.0
"upper"	275580	231734	61.0	90004	71506	75.5	39028	24015	5.6
"lower"									
Tables from where original data is recorded	2.1, 2.9, 2.11, 2.18	2.1, 2.8, 2.10, 2.16, 2.18	2.13, 2.14, 2.15, 2.16, 2.18	2.6, 2.11, 2.18	2.6, 2.10, 2.15, 2.18	2.13, 2.15, 2.18	2.7, 2.9, 2.18	2.7, 2.8, 2.16, 2.18	2.14, 2.16, 2.18

CHAPTER III.

Distribution of Carbon Dioxide Fixation Products in Roots in Relation to Flooding Tolerance.

In the previous Chapter, only quantitative changes in the total $C^{14}O_2$ -fixation of roots were examined. However, it was thought it would be of greater value to elucidate the distribution pattern of the labelled metabolites as well, in the search for a classification test for tree seedlings. Poel (1953) pointed out that in barley, the labelling of the $C^{14}O_2$ -fixation products of roots is greatly affected by changes in the pre-treatment of the plant material. Furthermore, it is known that an evaluation of the metabolic activity of the root system is possible by ascertaining the content of various organic and amino acids (Dubinina, 1962).

It is important to note that in this chapter, the data compared are expressed as percentages, if not otherwise stated. As in the previous chapter, the non-volatile 80 and 50 percent ethanol soluble compounds only are considered. Carbon dioxide incorporated into insoluble cellular material was not determined, and volatile compounds would not be detected due to their removal during the extraction procedure. Fats are extracted by the 80 percent ethanol; but a trace of them could be detected only in Alnus incana root. They were therefore excluded from the general survey given below. Further, the compounds containing fixed carbon dioxide may have been produced by exchange reactions or may represent a net synthesis. No attempt was made to determine the reactions with which each compound was formed.

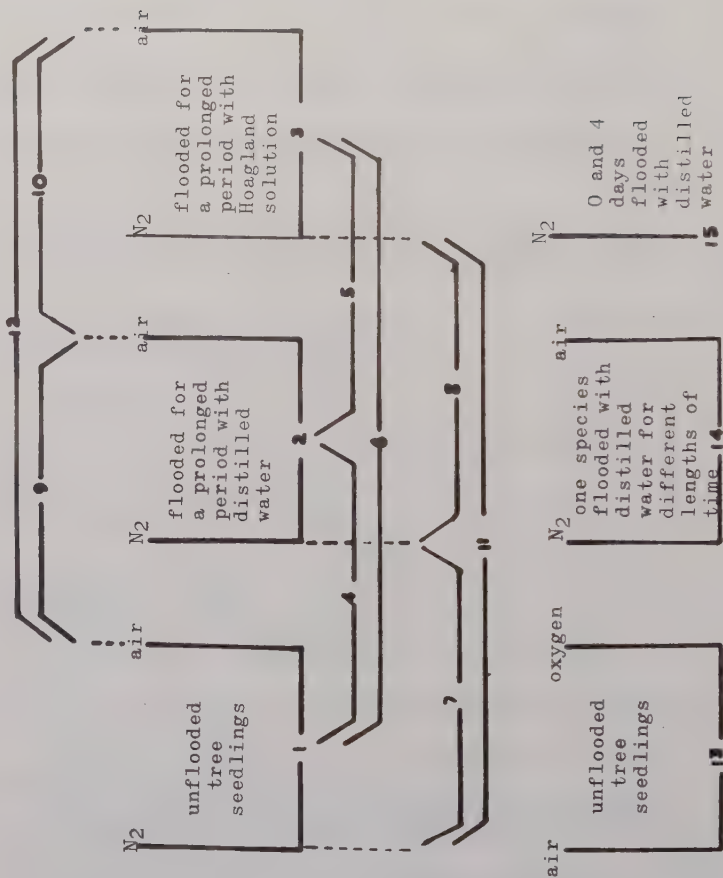
Finally, Fig. 3.1 illustrates the main aspects of flooding tolerance investigated in this Chapter. That is to say, not

Fig. 3.1. Diagram of different comparisons that are made between the numerous flooding treatments to elucidate the flooding tolerance by means of the distribution pattern of $C^{14}O_2$ -fixation products in roots of tree seedlings.

The treatments compared are:

- No.1. Unflooded plants, incubated under nitrogen, and then under air.
- No.2. As in No.1, except this time the plants have been flooded with distilled water for a prolonged period.
- No.3. As in No.1, except this time the plants have been flooded with Hoagland solution for a prolonged period.
- No.4. Plants of varying degrees of dependence on oxygen supply flooded for a prolonged period with distilled water, and then unflooded plants of the same species.
- No.5. As in No.4, except this time the plants which have been flooded for a prolonged period with distilled water are compared with those flooded for the same lengths of time in Hoagland solution.
- No.6. As in No.4, except this time unflooded control plants are compared with those flooded for a prolonged period with Hoagland solution.
- No.7. Plants flooded for a prolonged period with distilled water, and then plants not flooded, both being incubated under nitrogen.
- No.8. Plants flooded for a prolonged period with Hoagland solution compared with plants flooded for the same length of time in distilled water, both of which were incubated under nitrogen.
- No.9. As in No.7, except this time incubated under air.
- No.10. As in No.8, except this time incubated under air.
- No.11. Plants flooded for a prolonged period with Hoagland solution compared with unflooded control plants, both of which were incubated under nitrogen.
- No.12. As in No.11, except this time incubated under air.
- No.13. Unflooded plants, incubated under air, and then cylinder oxygen.
- No.14. A single species flooded with distilled water, but for different lengths of time, incubated under air, and under nitrogen.
- No.15. Plants flooded for four days with distilled water, and plants not flooded, both being uncubated under nitrogen.

Fig. 3.1. Diagram of different comparisons that are made between the numerous flooding treatments to elucidate the flooding tolerance by means of the distribution pattern of $C^{14}O_2$ -fixation products in root of tree seedlings.



only were the $C^{14}O_2$ -fixation products of roots compared when the tree seedlings were flooded with distilled water or Hoagland solution with unflooded controls, but also the oxygen dependence with or without an adaptation period was investigated.

1. The effect of oxygen supply on the distribution of the $C^{14}O_2$ -fixation products in roots of unflooded tree seedlings.

If in unflooded tree seedlings the total quantity of isotopes fixed by the roots into any one metabolite is compared under air and under nitrogen, as in Chapter 2.1 (see p.³¹), then it is only malic acid which shows a regular response to the different treatments. In unflooded tree seedlings, incubation under nitrogen causes a greater reduction in the label incorporated into malic acid in the non-tolerant seedlings than in the flood-tolerant species. This pattern of fixation in relation to flooding tolerance is shown in Table 3.1. It stresses the key position of malic acid in the metabolism, deriving from the $C^{14}O_2$ -fixation of roots.

Dubinina (1962) demonstrated that the degree of oxygen supply exerts a marked effect on their metabolism and postulated that under oxygen deficient conditions, a retardation of the Krebs cycle activity and a delay in the utilization of amino acids in protein synthesis took place. The present study (cf. Fig. 3.1, Comparison 1) shows that oxygen-deficiency not only reduces the total $C^{14}O_2$ -fixation of roots (Table 2.1) but also diminishes the percentages of labelled amino acids (except Larix laricina), carbohydrates and phosphate esters (Figs. 3.2 and 3.3).*

*N.B. For all substances other than malate, it is not the total counts fixed in any particular metabolite that are significant, rather the percentages of the total counts found in any one substance under the different treatments.

Table 3.1. Total amount of malic acid after a $C^{14}O_2$ fixation of roots of unflooded tree seedlings for one hour under nitrogen and air.

(a) Different species

	Air	N ₂	Ratio N ₂ /air
<i>Pinus contorta</i>	39,526	41,458	1.05
<i>Alnus incana</i>	116,456	94,361	0.81
<i>Larix laricina</i>	8,338	6,361	0.76
<i>Picea mariana</i>	56,635	34,223	0.60
<i>Picea sitchensis</i>	97,776	51,822	0.53
<i>Pinus sylvestris</i>	65,549	32,973	0.50
<i>Larix europea</i>	25,013	11,203	0.48

(b) Different provenances of *Picea mariana*

67063	56,635	34,223	0.60
5088	145,593	100,439	0.69

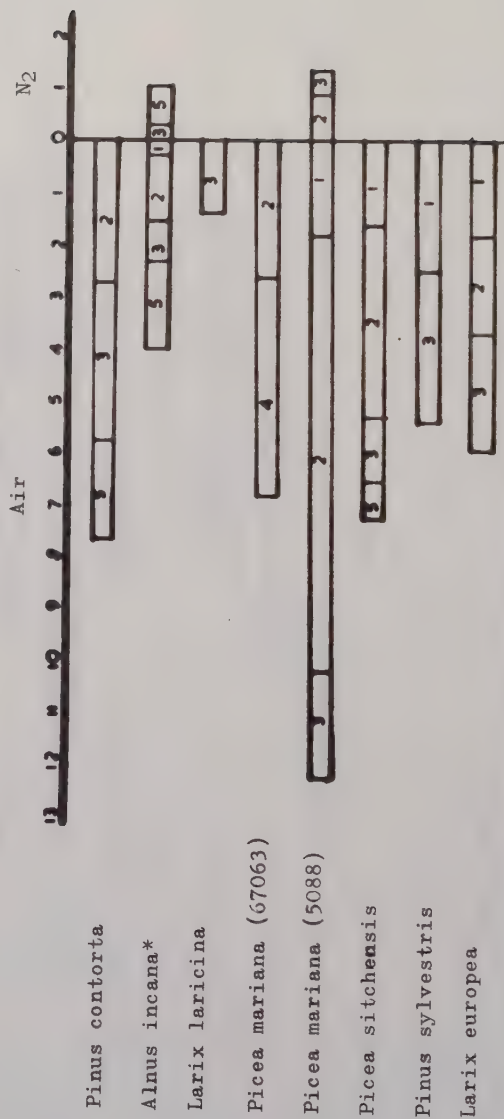
Fig. 3.2. Percentages of total radioactivity incorporated into amino acids after a $C^{14}O_2$ -fixation for one hour by roots of unflooded tree seedlings incubated under air and then under nitrogen. Each value is the mean of three measurements.

Species	AIR												M ₂															
	34	32	28	24	20	16	12	8	4	0	4	8	12	16	20	24												
<i>Pinus contorta</i>					4	3						2										1			2			3
<i>Alnus incana</i> **														7								1			2			12
<i>Larix laricina</i>																						2			2			3
<i>Picea mariana</i> (67063)																												
<i>Picea mariana</i> (5088)																												
<i>Picea sitchensis</i>																												
<i>Pinus sylvestris</i>																												
<i>Larix europea</i>																												

1. Aspartic acid, 2. glutamic acid, 3. alanine, 4. tyrosine, 5. glutamine, 7. citrulline.

** mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

Fig. 3.3. Percentages of total radioactivity incorporated into the carbohydrates and phosphate esters after a $C^{14}O_2$ -fixation for one hour by roots of unflooded tree seedlings incubated under air and then under nitrogen.



1. Phosphate ester(unknown), 2.UMP, 3.Sucrose, 5.Glucose.

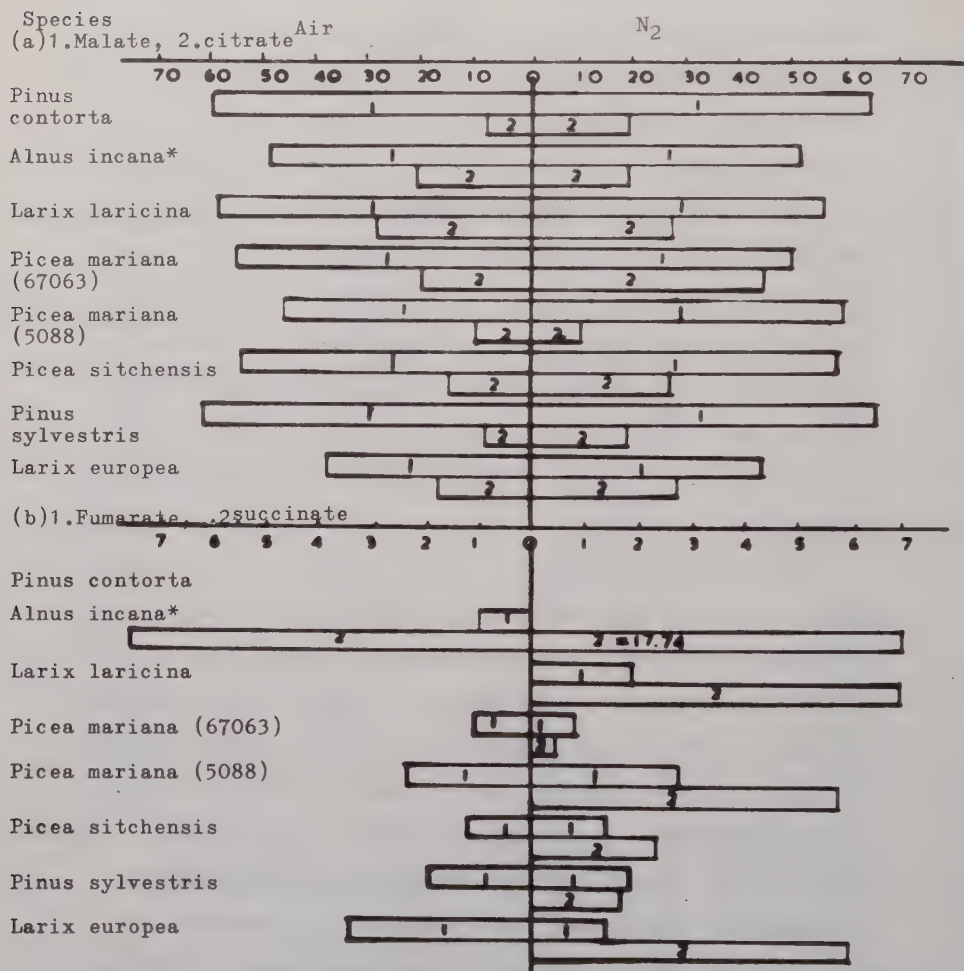
*means of samples from "upper" and "lower". (at least 17 cms below the soil surface) roots.

The improved turnover of labelled metabolites under air, noted above, might be due to an enhanced formation of NADH and ATP under air, or even to oxygen-dependent enzymes, as, for instance, phenylalanine hydroxylase. Fig. 3.2 verifies that in unflooded tree seedlings, tyrosine is found in countable amounts only if $C^{14}O_2$ is fixed under air. Furthermore, glutamic acid is always more highly labelled under than nitrogen, probably because its formation involves an oxidation of NADH. Through the synthesis of glutamic acid, most of NH_4^+ enters into the organic cell-metabolism. Thus, this is the key reaction for the whole NH_4^+ -assimilation in cells. A smaller part of NH_4^+ -assimilation can also be contributed through glutamine, which might be formed from glutamic acid with ATP. Therefore it is of note that glutamine is found labelled only when roots are incubated under air.

There is no distinctive pattern in relation to oxygen supply regarding the labelling of aspartic acid and citrulline. However, attention should be drawn to the fact that under nitrogen, the labelling of alanine always increases. It might be formed by a transaminase reaction from pyruvate without ATP, where glutamic acid would be the NH_2 -donator. As this reaction does not require ATP, it can continue unrestrictedly under anaerobic conditions. The resulting α -ketoglutaric acid seems to be converted immediately to fumaric acid. Under nitrogen, an enhanced labelling of this acid is always detected (Fig. 3.4).

In addition, it should be pointed out that under air and nitrogen, malic acid is always the most highly labelled product of $C^{14}O_2$ -fixation, and that the sum of labelled

Fig. 3.4. Percentages of total radioactivity incorporated into organic acids after a $C^{14}O_2$ -fixation for one hour by roots of unflooded tree seedlings incubated under air, and then under nitrogen. Each value is the mean of three measurements.



*means of samples from "upper" and "lower" (at least 17 cms below the soil surface) roots.

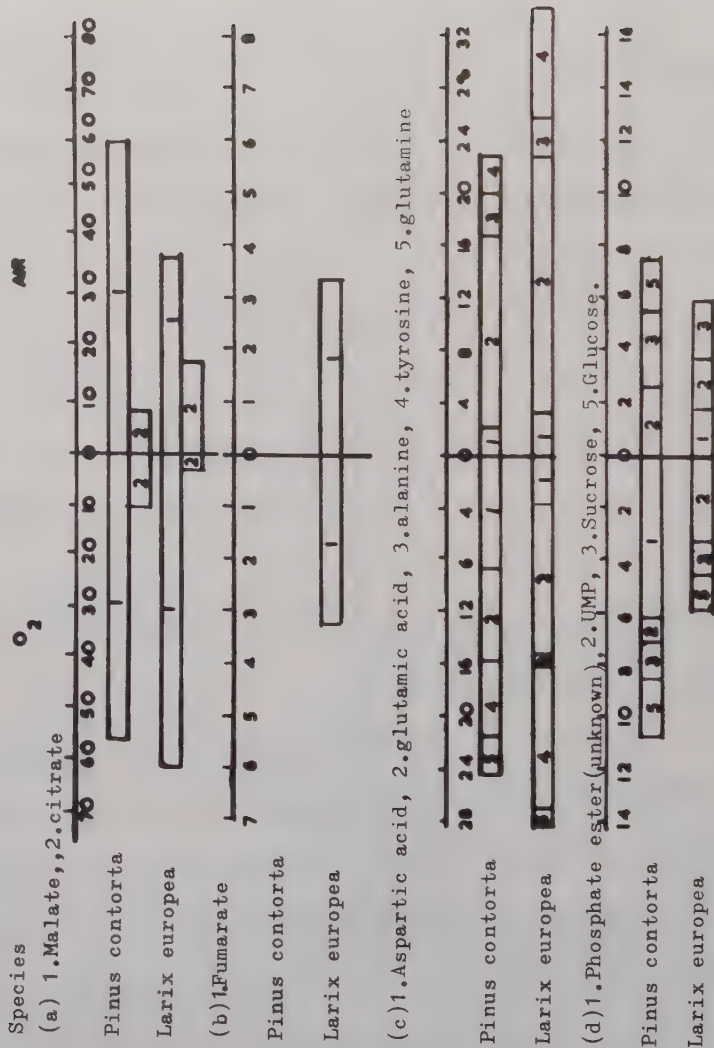
succinic and fumaric acid is 1/10 of that found in malic and citric acids. This would indicate that in roots the metabolism of $C^{14}O_2$ -fixation products is never rapid, since the label is only slowly dispersed into the general metabolic pool.

Fig. 3.5 compares the distribution of the $C^{14}O_2$ -fixation products of roots under air and cylinder oxygen (cf. Fig. 3.1 Comparison 13). Noteworthy, is the fact that the labelling of glutamic acid and tyrosine is enhanced under cylinder oxygen, whereas the labelling of aspartic acid and alanine decreases under these conditions. This seems to confirm the above interpretation that the relative labelling of alanine decreases with increased aeration because its formation by a direct amination does not involve ATP. As well, the enzyme phenylalanine hydroxylase (Karlson 1966) seems to be of importance for the labelling of tyrosine, and it seems that there is still more NADH available under cylinder oxygen than under air to enhance the labelling of glutamic acid.

Earlier experiments by Betz (1957) and Berry(1949) (see also Kandler, 1958) demonstrated that the meristem region of roots respire with a R.Q. value greater than one, leading to the production of ethanol, even under conditions of good aeration. These authors found that it required incubation under high partial pressures of oxygen to remove this anaerobic fermentation effect. These results are similar to the effects noted in the present study, where the increased labelling of alanine under anaerobic conditions is progressively reduced when the roots are placed under high concentrations of oxygen.

Finally it must be pointed out that the method of root sampling with Alnus incana differed from that used with all

Fig. 3.5. Percentages of total radioactivity incorporated into $C^{14}O_2$ -fixation products after one hour by roots of unflooded tree seedlings incubated under cylinder oxygen, and then under air. Each value is the mean of three measurements.

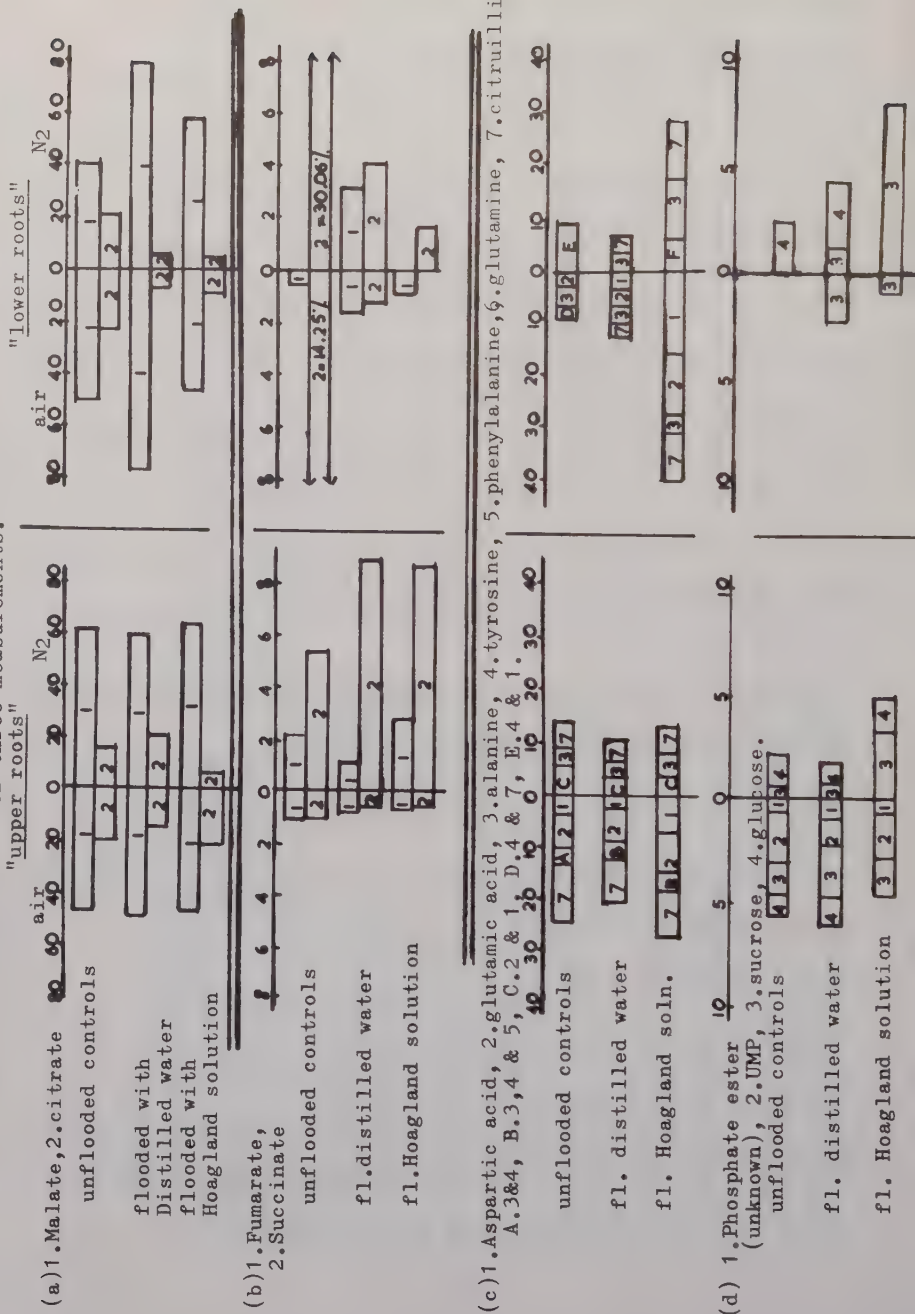


other trees. With alder, the trees were much larger, and the entire rooting system could not be taken for the incubation treatments. Instead, an upper and lower root-sample was taken from pot-grown three to four year old trees. The lower layer was always taken at a depth of more than 17 cms. Equal amounts were taken from these layers. The results presented so far are the means of the two samples. In this way, it was hoped that the distribution of young and old root-tissue in the alder sample matched that of the other trees. However, Fig. 3.6 gives the values obtained for "lower" (at least 17 cms below the soil surface) and "upper" roots separately. It was observed that the labelling of succinic acid is highly enhanced and the labelling of amino acids, phosphate esters and carbohydrates is reduced in the "lower" roots, when compared with the roots near the soil surface.

2. Effect of waterlogged soil conditions on the distribution of the $C^{14}O_2$ -fixation products of roots.

The results previously discussed reveal that the $C^{14}O_2$ -fixation of roots and their products are greatly affected by changes in the oxygen supply, even when the tree seedlings have not been pretreated. The results represented below (Figs. 3.6 and 3.7 - 3.11) summarize all data obtained, when tree seedlings have been pretreated by flooding with distilled water. The different aspects of flooding tolerance discussed in this paragraph are illustrated in Fig. 3.1. In this figure, the different comparisons that are made between the numerous flooding treatments are summarized in diagrammatic form. The treatments compared in this paragraph are:-

Fig. 3.6. Percentages of total radioactivity incorporated into $C^{14}O_2$ -fixation products after one hour by *Alnus incana* roots incubated under air, and then under nitrogen. Each value is the mean of three measurements.

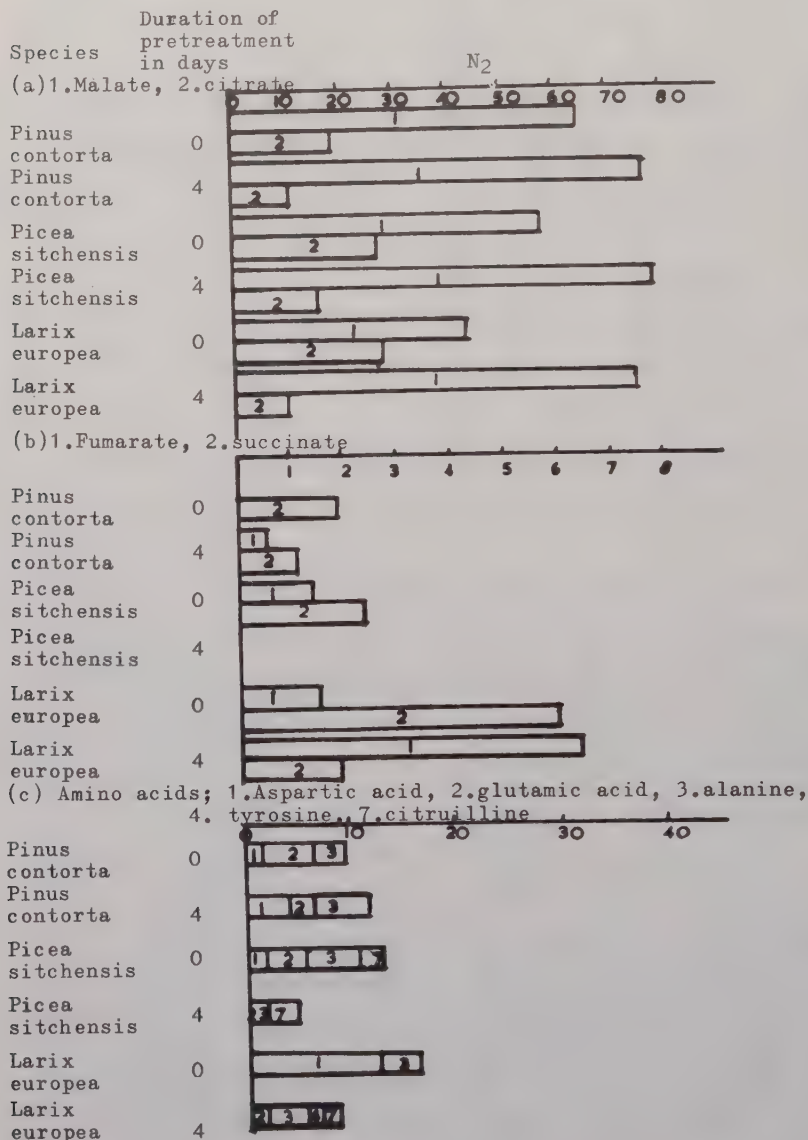


- No.2. Plants flooded with distilled water for a long period under nitrogen, and then air.
- No.4. Plants of varying degrees of dependence on oxygen supply flooded for a prolonged period with distilled water, and unflooded plants of the same species.
- No.7. Plants flooded for a prolonged period with distilled water, and plants not flooded, both being incubated under nitrogen.
- No.9. The same as in No.7, except this time incubated under air.
- No.14. A single species flooded with distilled water, but for different lengths of time, incubated under air, and under nitrogen.
- No.15. Plants flooded for four days with distilled water, and plants not flooded, both being incubated under nitrogen.

Not all metabolites are equally affected by flooding. In this section, an account is given mainly of those substances which showed a distinctive labelling pattern as a result of a flooding treatment, and of those aspects which reveal a classification test of flooding-tolerance according to the sequence of flooding-tolerance obtained in the previous Chapters.

In Fig.3.7 the flooding-tolerant species Pinus contorta is compared with the non flood-tolerant species Picea sitchensis and Larix europea (cf. Fig. 3.1, Comparison 15). The data show the percentages of labelled compounds after a fixation period under nitrogen for one hour, when the tree seedlings have been flooded for four days with distilled water. After a short flooding period malic, citric and succinic acids reveal the same pattern in all species used.

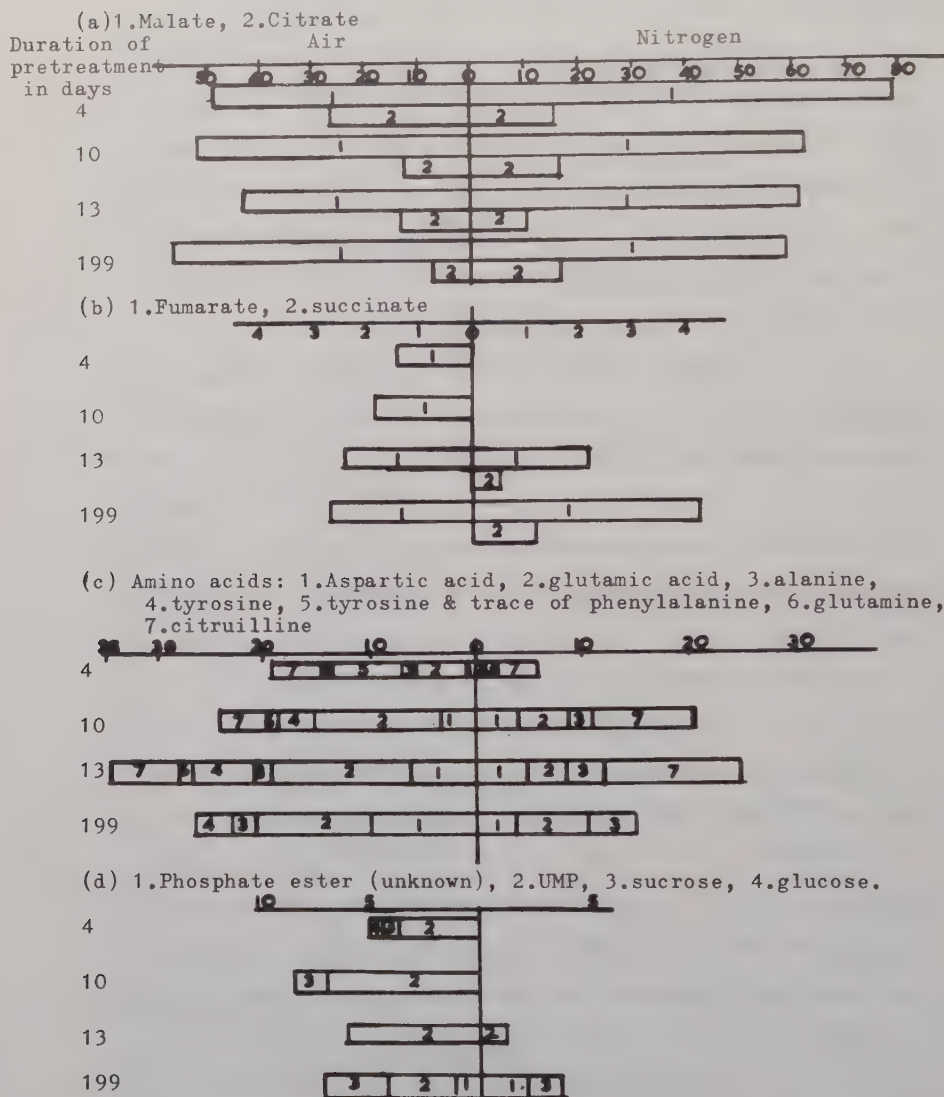
Fig. 3.7. Percentages of total radioactivity incorporated into $C^{14}O_2$ -fixation products by roots after one hour when the tree-seedlings have been flooded for four days with distilled water, and not flooded, both being incubated under nitrogen. Each value is the mean of three measurements.



In other words, malic acid increases, while citric and succinic acids decrease. This seems to indicate that the turnover of malic acid slows down in all pretreated seedlings. However, the comparison of changes in the distribution of labelled amino acids distinguishes Pinus contorta from the flood-intolerant species. The sum of the radio-activity incorporated into the amino acids, and in particular aspartic acid, increases in Pinus contorta after a short flooding treatment, while it decreases in the flood intolerant species. Also it was noted that labelled citrulline could not be detected in Pinus contorta(flood-tolerant), which contrasts with the flood-intolerant species.

Fig. 3.8 summarizes the results obtained with Picea sitchensis roots, when the seedlings have been flooded with distilled water for four, ten, thirteen and a prolonged period of 199 days (cf. Fig. 3.1, Comparison 14). Under nitrogen, the labelling of malic acid decreases, whereas fumaric and succinic acids increases with every increase in the time intervals for the flooding treatment. This could indicate an enhanced turnover rate of the labelled metabolites in roots in relation to the duration of the pretreatment. The finding that the labelling of the phosphate esters, sucrose and alanine similarly increases, strengthens that possibility. But the labelling of citrulline was found to increase only under short term flooding treatments, viz. 0 - 13 days, and could not be detected after a long term flooding treatment. This could well indicate that the seedlings of Picea sitchensis, a non-flood-tolerant species whose seedlings can only just survive a long term treatment with distilled water, show a correlation between normal growth and a CO₂-fixation in roots into citrulline. Nonetheless, this additional CO₂-fixation

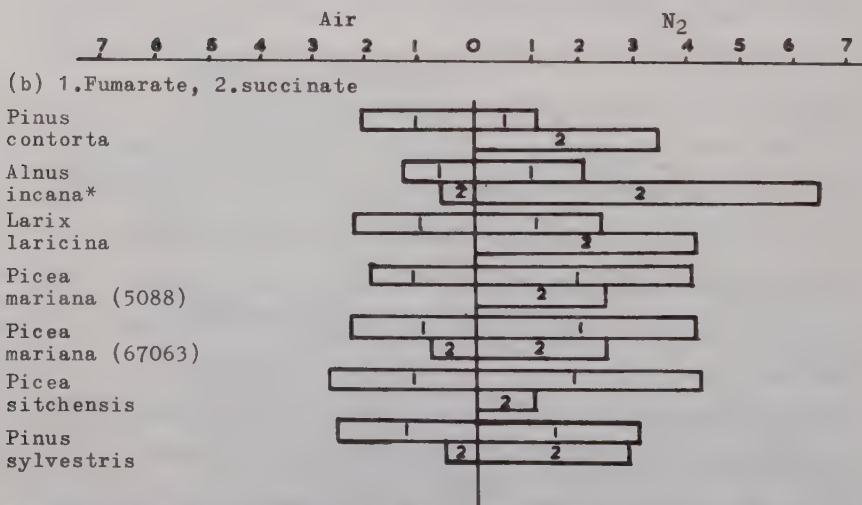
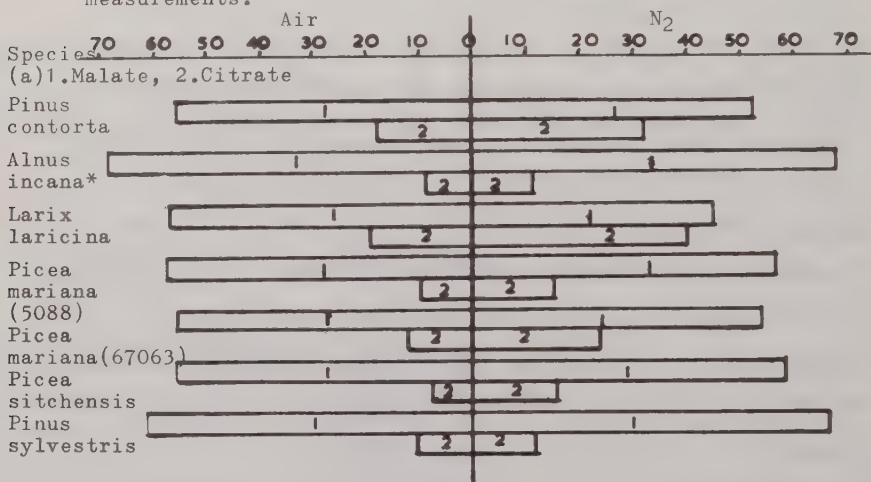
Fig. 3.8. Percentages of total radioactivity incorporated into $C^{14}O_2$ -fixation products of *Picea sitchensis* roots incubated under air, and then under nitrogen. The seedlings have been flooded for different lengths of time. Each value is the mean of three measurements.



seems to disappear after a prolonged period of treatment under waterlogged soil conditions. If the $C^{14}O_2$ -fixation of roots takes place under air, then the labelling of citrulline shows the same pattern of an initial increase followed by a decrease with prolonged flooding. Also, after a long-term pretreatment, the labelling of glutamine ceases and that of tyrosine decreases. These changes may be a sign of a reduced concentration of ATP in the cells of Picea sitchensis roots, when the tree seedlings have been flooded with distilled water for a prolonged period. Finally, the labelling of glutamic acid increases under air in relation to the duration of the flooding treatment, whereas labelling of aspartic acid increases only in seedlings flooded for a short period (0 - 13 days).

All further results represented in the Chapter are obtained only from tree seedlings after they have been flooded for a prolonged period. These results are examined from the same standpoint as illustrated in Fig. 3.1, Comparison 2. Fig. 3.9 gives the percentages of labelled organic acids after a $C^{14}O_2$ -fixation of roots under air and nitrogen when the tree seedlings have been flooded for a prolonged period with distilled water. The malic and citric acids are again more highly labelled than the fumaric and succinic acids. Taken together, the percentages of labelled citric and succinic acids are higher after a fixation under nitrogen than under air. This seems to indicate that in roots of long-term flooded seedlings, the dispersion of the label is retarded under nitrogen, as was found for the unflooded seedlings. It was recorded that under nitrogen, the labelling of succinic acid increases more in the flood-tolerant species

Fig. 3.9. Percentages of total radioactivity incorporated into the organic acids after a $C^{14}O_2$ -fixation for one hour by roots under air, and then under nitrogen. The seedlings have been flooded for a prolonged period with distilled water. Each value is the mean of three measurements.



*means of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

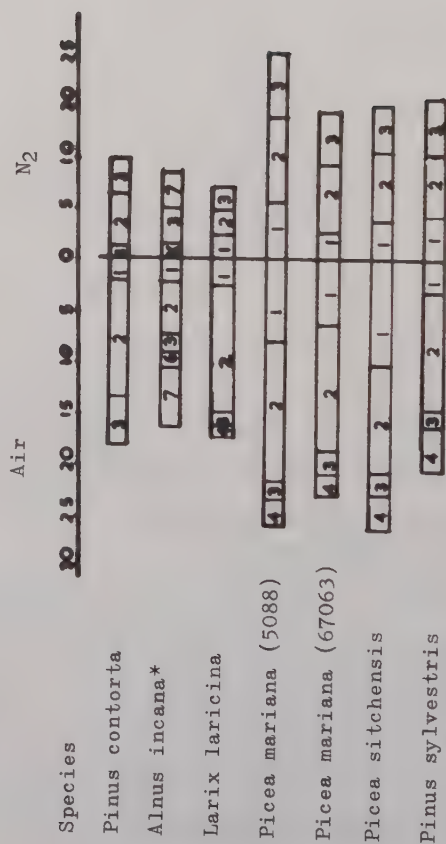
than in the flood-intolerant plants.

The percentages of labelled amino acids under air and nitrogen are summarized in Fig. 3.10 where the tree seedlings have been flooded for a prolonged period with distilled water. This shows not only that the labelling of amino acids is enhanced under air, but also that under air and nitrogen the labelled amino acids are less in the flood-tolerant species than in the non flood-tolerant seedlings.

It is of interest that in the flood-tolerant species, the labelling of succinic acid and the amino acids under nitrogen is the reverse of that found in the flood-intolerant plants. These results suggest that in the flood-tolerant species, the amination-reactions are slowed down under nitrogen as compared with the non-tolerant plants. This might be due to a comparatively lower rate of deproteinisation in the flood-tolerant species. This behaviour, when the plants are flooded in distilled water, must be contrasted with that found when the plants are flooded in Hoagland solution. Here flood-tolerant species show an increase in the label incorporated into the amino acids. (For a fuller account, see Discussion, pp.88 and 89).

Fig. 3.11 summarizes the labelled carbohydrates and phosphate esters, when the tree seedlings have been pretreated for a prolonged period with distilled water. As in all cases so far discussed, the incorporation of C^{14} is enhanced in these compounds under air. Alnus incana roots are the only material to yield countable amounts of labelled carbohydrates and phosphate esters under nitrogen.

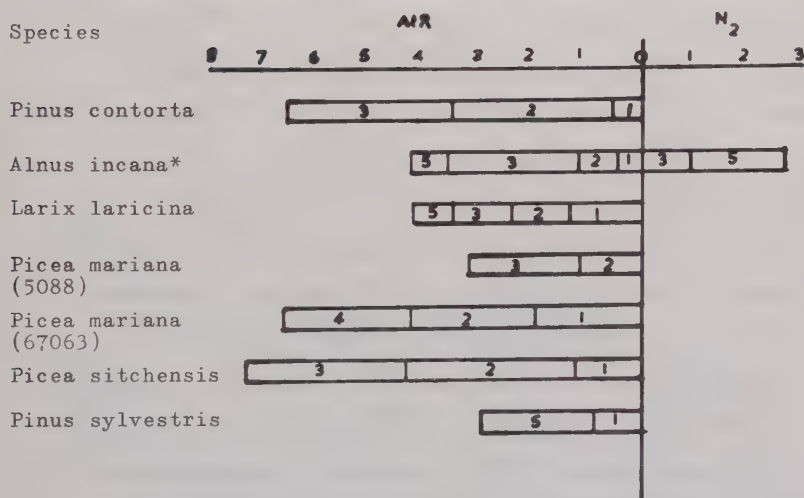
Fig. 3.10. Percentages of total radioactivity incorporated into the amino acids after a $C^{14}O_2$ -fixation for one hour by roots under air, and then under nitrogen. The seedlings have been flooded for a prolonged period with distilled water. Each value is the mean of three measurements.



*means of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

1. Aspartic acid, 2. glutamic acid, 3. alanine, 4. tyrosine, 5. glutamine, 6. phenylalanine and tyrosine, 7. citrulline, x. aspartic acid and glutamic acid.

Fig. 3.11. Percentages of total radioactivity incorporated into the carbohydrates and phosphate esters after a $C^{14}O_2$ -fixation for one hour by roots under air, and then under nitrogen. The seedlings have been flooded for a prolonged period with distilled water. Each value is the mean of 3 measurements.



1. Phosphate esters (unknown), 2.UMP, 3.Sucrose, 4.Sucrose and Maltose, 5.Glucose.

* means of samples from "upper" and "lower"(at least 17 cms below the soil surface) roots.

It must be mentioned that the values for Alnus incana, cited in this paragraph, are means. Tet Fig. 3.6b illustrates that in pretreated plants, the "lower" roots (at least 17 cm below the soil surface) show a higher labelling in the malic and fumaric acids than the "upper" roots, and the reverse for the labelling of amino acids, carbohydrates and phosphate esters. This seems to indicate that after a prolonged flooding period with distilled water, the turnover rate of labelled $C^{14}O_2$ -fixation products is reduced in the "lower" roots, as compared to the roots near the soil surface.

So far we have only discussed the dependence of the labelled $C^{14}O_2$ -fixation products of the roots on oxygen supply, after long periods of flooding. Yet we may also compare plants flooded for a prolonged period in distilled water, and unflooded plants of the same species in relation to the oxygen-dependence of the species. (cf. Fig. 3.1, Comparison 4). In flood-tolerant species, the labelling of the aspartic and glutamic acids, taken together, decreases relatively more under nitrogen than under air, after a prolonged period of flooding with distilled water. That is to say, the ratio of fixation in nitrogen : air decreases in flood-tolerant species after a prolonged flooding period (Table 3.2). This seems to indicate that in pretreated seedlings of flood-tolerant species, the deproteinisation, which might be responsible for the amination of the labelled organic acids, slows down more under nitrogen than under air.

However, in the flood-intolerant species Picea sitchensis and Pinus sylvestris, an increase of the percentages of the labelled aspartic and glutamic acids (taken together), was

Table 3.2. The effect of anaerobiosis on the labelling of aspartic and glutamic acid as seen in the ratio Nitrogen:Air of the percentage label found in root extracts after a one hour fixation period in the relevant atmosphere. Unflooded tree seedlings are compared with those that have been flooded for a prolonged period with distilled water.

(a) Different species

Species	Ratio N ₂ :Air	
	Unflooded controls	Flooded in Distilled Water
<i>Pinus contorta</i>	0.70	0.17
<i>Alnus incana</i>	0.54	0.49
<i>Larix laricina</i>	1.24	0.30
<i>Picea mariana</i>	0.47	0.45
<i>Picea sitchensis</i>	0.36	0.49
<i>Pinus sylvestris</i>	0.38	0.70

(b) Different provenances of *Picea mariana*

5088	0.75	0.76
67063	0.47	0.45

observed under nitrogen, when tree seedlings have been flooded for a prolonged period, in contrast to the unflooded control plants. It thus seems that in flood-intolerant species, the amination of organic acids is enhanced under anaerobic conditions. For when we compare the results obtained for the two varieties of Picea mariana (5088 and 67063), when flooded and not flooded, the sum of the percentages of the labelled aspartic and glutamic acids shows no changes, as regards their dependence on oxygen supply.

Regarding Fig. 3.1, it becomes apparent that there are still two possibilities in comparing the results obtained (Comparison 7 and 9). Firstly, the distribution under nitrogen of some labelled metabolites of tree seedlings, after they have been flooded for a prolonged period with distilled water, is compared to that in unflooded tree seedlings (Fig. 3.1, Comparison 7). The percentages of labelled citric acids show a difference in relation to the flooding-tolerance of the plant material used (Table 3.3). In flood-intolerant species, the percentages of labelled citric acid decrease, while they increase in the flood-tolerant plants, due to a prolonged flooding period with distilled water. Even the two groups of Picea mariana examined follow the same pattern. The two provenances of Picea mariana used varied in their flooding-tolerance. The material from a dry sandy plain (5088) was more flood-tolerant (when treated with distilled water) than the 67063 provenance. The latter provenance, Picea mariana 67063, not only suffers severely in distilled water, but also the roots of the plants of this group, which still

Table 3.3. Percentages of labelled citric acid after a $C^{14}O_2$ -fixation of roots under nitrogen for one hour when the tree seedlings have been flooded for a prolonged period with distilled water, compared with unflooded control plants.

(a) Different species

Species	Control unflooded	Flooded in distilled water	Ratio Flooded: unflooded
<i>Pinus contorta</i>	18.44	32.73	1.78
<i>Alnus incana</i>	18.68	21.38	1.15
<i>Larix laricina</i>	25.98	40.22	1.55
<i>Picea mariana</i>	43.48	24.26	0.56
<i>Picea sitchensis</i>	20.05	16.87	0.84
<i>Pinus sylvestris</i>	20.93	12.27	0.59

(b) Different provenances of *Picea mariana*

5088	9.84	16.01	1.63
67063	43.48	24.26	0.56

appear healthy, reveal lower percentages of labelled citrate when they have been flooded for a prolonged period.

Furthermore, under nitrogen, the labelled amino acids distinguish between flood-tolerant and intolerant species, including the two groups of Picea mariana (Table 3.4). After a long term flooding treatment, the percentages of labelled amino acids decrease in the flood-tolerant species, while they increase in the flood-intolerant seedlings. Tables 3.5 - 3.7 reveal the same pattern as well with the alanine, aspartic, and glutamic acids. The labelling of glutamic acid is especially noteworthy. The ratio of labelled glutamic acid in flooded and unflooded seedlings yields a clear relation to the sequence of the flooding-tolerance of the species used (cf. Chapter 1, Table 1.17, and Chapter 2, Table 2.1). In other words, the flooding-tolerance of the species used increases alongside the decreased labelling of glutamic acid, after a prolonged pretreatment with distilled water.

With Picea mariana, the nature of the flooding solution is of overriding importance in relation to its flooding-tolerance. Plants of the provenance number 5088 are intolerant of flooding in Hoagland solution, while those of the provenance number 67063 suffer more in distilled water. If the distilled water treatment is taken as the flooding tolerance test, then the pattern of $C^{14}O_2$ labelling corresponds to the general behaviour found in the other species tested.*

* N.B. Hitherto the species have been listed in decreasing order of flooding-tolerance, as based on the growth tests in Chapter I. In these tests, Picea mariana 67063 was more tolerant of flooding than Picea mariana 5088. However, it is apparent that in prolonged flooding experiments with distilled water, the latter strain (5088) is the more tolerant of the two. Therefore in all the Tables 3.2 to 3.8 provenance 5088 is tested before 67063 (the reverse of the previous order).

Table 3.4. Percentages of labelled amino acids after a $C^{14}O_2$ -fixation of roots under nitrogen for one hour when the tree seedlings have been flooded for a prolonged period with distilled water as compared with unflooded control plants.

(a) Different species

Species	Unflooded controls	Flooded in distilled water	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	15.74	9.51	0.60
<i>Alnus incana</i>	11.10	8.65	0.78
<i>Larix laricina</i>	10.30	7.00	0.68
<i>Picea mariana</i>	6.75	14.21	2.11
<i>Picea sitchensis</i>	10.26	14.94	1.46
<i>Pinus sylvestris</i>	9.84	15.11	1.54

(b) Different provenances of *Picea mariana*

5088	21.16	20.05	0.95
67063	6.75	14.21	2.11

Table 3.5. Percentages of labelled aspartic acid after a $C^{14}O_2$ -fixation of roots under nitrogen for one hour when the tree seedlings have been flooded for a prolonged period with distilled water compared with unflooded control plants.

(a) Different species

Species	Unflooded controls	Flooded in distilled water	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	2.22	1.43	0.64
<i>Alnus incana</i>	0.98	0.53	0.54
<i>Larix laricina</i>	2.57	2.17	0.84
<i>Picea mariana</i>	1.52	2.14	1.41
<i>Picea sitchensis</i>	1.58	3.82	2.42
<i>Pinus sylvestris</i>	2.54	4.22	1.66

(b) Different provenances of *Picea mariana*

5088	4.54	5.50	1.21
67063	1.52	2.03	1.41

Table 3.6. Percentages of labelled alanine after a $C^{14}O_2$ -fixation of roots under nitrogen for one hour when the tree seedlings have been flooded for a prolonged period with distilled water compared with unflooded control plants.

(a) Different species.

Species	Unflooded controls	Flooded in Distilled Water	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	5.01	3.14	0.63
<i>Alnus incana</i>	5.45	3.18	0.58
<i>Larix laricina</i>	3.44	2.36	0.69
<i>Picea mariana</i>	2.06	5.20	2.52
<i>Picea sitchensis</i>	3.26	4.57	1.40
<i>Pinus sylvestris</i>	4.20	4.74	1.13

(b) Different provenances of *Picea mariana*

5088	9.64	6.22	0.65
67063	2.06	5.20	2.52

Table 3.7. Percentages of labelled glutamic acid after a $C^{14}O_2$ -fixation of roots under nitrogen for one hour when the tree seedlings have been flooded for a prolonged period with distilled water compared with unflooded control plants.

(a) Different species

Species	Unflooded controls	Flooded in Distilled water	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	9.59	4.94	0.52
<i>Alnus incana</i>	5.28	2.87	0.54
<i>Larix laricina</i>	4.27	2.47	0.58
<i>Picea mariana</i>	3.17	6.87	2.17
<i>Picea sitchensis</i>	2.33	6.55	2.81
<i>Pinus sylvestris</i>	1.86	6.15	3.31

(b) Different provenances of *Picea mariana*

5088	4.54	5.50	1.21
67063	3.17	6.87	2.17

This finding emphasizes not only the key position of glutamic acid in the NH_4 -assimilation of the cells, but also reveals a new possibility for the determination of the relative flood-tolerance of tree seedlings. Furthermore, it must be pointed out that in flood-intolerant species under anaerobic conditions, the percentages of labelled amino acids increase after a prolonged pretreatment, in contrast to the unflooded plants (Fig. 3.1, Comparison 4). As well, there seems to be an enhanced amination of organic acids in roots of long-term flooded seedlings under anaerobic conditions, in comparison to the distribution of labelled products under air (Fig. 3.1, Comparison 6). Flood-tolerant species, however, show the reverse pattern. It would thus seem that in flood-tolerant species, the deproteinisation reactions slow down under anaerobic conditions.

Finally, the distribution of labelled C^{14}O_2 -fixation products of roots under air is of interest, when tree seedlings have been flooded for a prolonged period with distilled water, and the unflooded control plants are compared (cf. Fig. 3.1, Comparison 9). Under air, the amount of label in tyrosine is of particular note. The labelling of tyrosine decreases with the flooding pretreatment in the flood-tolerant species, whereas it increases in the flood-intolerant seedlings (Table 3.8). All other possibilities to compare the labelled compounds under air, when seedlings have been flooded for a prolonged period with distilled water and grown unflooded, do not reveal any regular pattern or relationship to the flooding-tolerance of the species used.

Table 3.8. Percentages of labelled tyrosine after a $C^{14}O_2$ -fixation of roots under air for one hour when tree seedlings have been flooded for a prolonged period with distilled water compared with unflooded control plants.

(a) Different species.

Species	Unflooded controls	Flooded in distilled water	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	3.40	-	-
<i>Alnus incana</i>	1.23	0.86	0.70
<i>Larix laricina</i>	4.54	0.92	0.20
<i>Picea mariana</i>	0.63	2.25	3.57
<i>Picea sitchensis</i>	2.58	3.54	1.37
<i>Pinus sylvestris</i>	3.90	4.39	1.13

(b) Different provenances of *Picea mariana*

5088	6.25	2.54	0.41
67063	0.63	2.25	3.57

3. Effect of additional nutrients on the distribution of $C^{14}O_2$ -fixation products in roots in relation to flooding tolerance.

It was already pointed out in the previous Chapters that the use of Hoagland solution for experimental flooding has the fertilizer effect normally expected only in the flood-tolerant species, while in flood-intolerant species, additional nutrients increase the mortality of the tree-seedlings. Poel (1953) suggested that the ethanol soluble fraction of barley roots, placed for 24 hours after excision in aerated tap-water or placed in Hoagland solution, might indicate that the transformation of C^{14} is impeded as a direct or indirect result of mineral starvation. The distribution pattern of labelled metabolites deriving from a $C^{14}O_2$ -fixation of roots was analysed in this present study after the tree seedlings have been flooded for a prolonged period with Hoagland solution. This was to check not only if Poel's results can also be obtained with tree seedlings, but also to elucidate if there are any further differences between flood-tolerant and intolerant tree species. All the results discussed subsequently were obtained both from plants which have been flooded for a prolonged period and from unflooded control plants. Fig. 3.1 illustrates all the relevant possibilities that can be compared, that is to say, the comparison numbers 3, 5, 6, 8, and 10 - 12:-

- No. 3. Plants flooded with Hoagland solution for a prolonged period, incubated under nitrogen, and under air.
- No. 5. Plants of varying degrees of dependence on oxygen-supply flooded for a prolonged period with Hoagland solution, as well as plants which have been flooded for the same

length of time with distilled water.

No. 6. As in No. 5, except this time unflooded control plants are compared with those flooded for a prolonged period with Hoagland solution.

No. 8. Plants flooded for a prolonged period with Hoagland solution compared with plants flooded for the same length of time with distilled water, both of which are incubated under nitrogen.

No. 10. As in No. 8, except this time incubated under air.

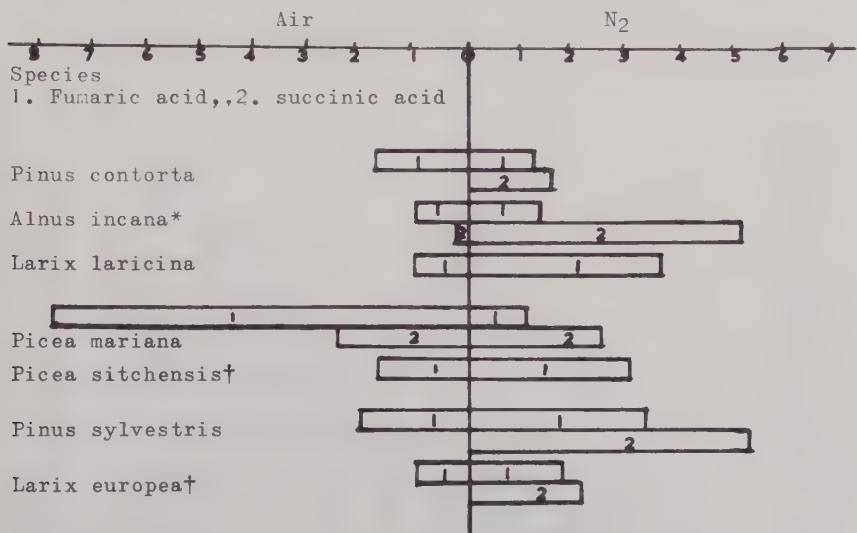
No. 11. Plants flooded for a prolonged period with Hoagland solution compared to unflooded control plants, both of which were incubated under nitrogen.

No. 12. As in No. 11, except this time incubated under air.

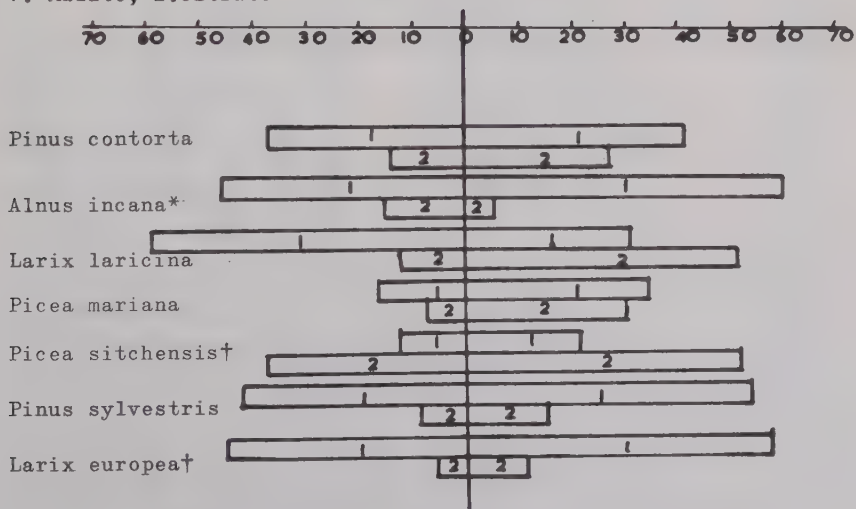
Figs. 3.12 - 3.15 give the data obtained for all labelled metabolites in roots, when the tree seedlings have been flooded for a prolonged period with Hoagland solution. It becomes apparent that the sum of the percentages of labelled organic acids decreases (Fig. 3.12) and that the labelling of the amino acids, in particular of glutamic acid, increases (Fig. 3.14) under air in all species used, in contrast to incubation under nitrogen. However, none of the other possibilities compared show any regular difference even between flood-tolerant and intolerant species in relation to the dependence of metabolites labelling on oxygen supply. Yet, it seems that in roots of seedlings which have been flooded for a prolonged period with Hoagland solution, the turnover of labelled metabolites is also increased under air, as has been found both with seedlings which have not been flooded and with those which have been pretreated with distilled water.

The comparison of seedlings which have been flooded for

Fig. 3.12. Percentages of total radioactivity incorporated into the organic acids after a $C^{14}O_2$ -fixation for one hour by roots under air, and nitrogen. The seedlings have been flooded for a prolonged period with Hoagland solution. Each value is the mean of three measurements.



1. Malate, 2.citrate



*means of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots

† means of two samples.

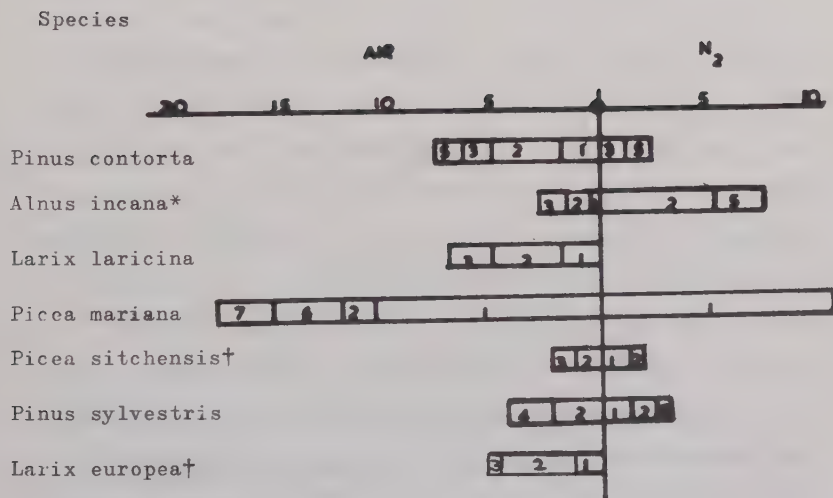
Fig. 3.13. Percentages of total radioactivity incorporated into the amino acids after a $C^{14}O_2$ -fixation for one hour by roots under air, and then under nitrogen. The seedlings have been flooded for a prolonged period with Hoagland solution. Each value is the mean of three measurements.

Species	Air											N ₂										
	50	45	40	35	30	25	20	15	10	5	0	5	10	15	20	25	30	35	40	45	50	
<i>Pinus contorta</i>				4	3		2			1		1		3		4	5					
<i>Alnus incana</i> *					7		11	2		1		1	2	3		7						
<i>Larix laricina</i>							13	2				1	1	1	2	3						
<i>Picea mariana</i>	4	3				2			1			1				2						
<i>Picea sitchensis</i> †		4	7			2			1			1		2		3						
<i>Pinus sylvestris</i>	5			4		3	2		1			1		2	3	4						
<i>Larix europae</i> †		4	3			2				1		1		2		3	4					

* means of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.
 † means of two samples

1. Aspartic acid, 2. glutamic acid, 3. alanine, 4. tyrosine, 5. glutamine, 6. phenylalanine & tyrosine, 7. citrulline.

Fig. 3.14. Percentages of total radioactivity incorporated into carbohydrates and phosphhte esters after a $C^{14}O_2$ -fixation for one hour by roots under air, and then under nitrogen. The seedlings have been flooded for a prolonged period with Hoagland solution. Each value is the mean of three measurements.



*means of sample from "upper" and "lower" (at least 17 cms below soil surface) roots.

† means of two samples.

1. Phosphate ester (Unknown), 2.UMP, 3. sucrose, 4.sucrose and maltose, 5. glucose, 6. maltose and fructose.

a prolonged period with Hoagland solution and distilled water, is thought to elucidate the effect of additional nutrients on the $C^{14}O_2$ -fixation of roots (cf. Fig. 3.1, Comparison 5). A characteristic of flood-tolerant species is that the labelling of organic acids is almost as oxygen dependent after a prolonged pretreatment with Hoagland solution as it was with distilled water (Table 3.9). However, the flood-intolerant species reveal a more marked decrease in the percentages of labelled organic acids, under air than under nitrogen, when flooded for a prolonged period with Hoagland solution, as compared with seedlings flooded in distilled water. This results in an increase in the nitrogen:air ratio for organic acids. In other words, in flood-intolerant pretreated seedlings, the labelling of metabolites becomes more dependent on oxygen supply in the presence of nutrients. In addition, a comparison of unflooded tree seedlings with those which have been flooded for a prolonged period with Hoagland solution proved to be of interest (cf. Fig. 3.1, Comparison 6). With regard to aspartic and glutamic acid, a clear difference between the flood-tolerant and intolerant seedlings becomes apparent (Table 3.10). That is to say, in the flood-tolerant species the labelling of aspartic and glutamic acids increases more under air than under nitrogen, when the seedlings have been flooded for a prolonged period with Hoagland solution, in contrast to the unflooded control plants. The reverse was found for the flood-intolerant species. In other words, the dependence on oxygen availability decreased in the flood-intolerant species.

Table 3.9. The effect of anaerobiosis on the labelling of all organic acids as seen in the ratio Nitrogen:Air of the percentage lable found in root extracts after a one hour fixation period in the relevant atmosphere. Tree seedlings which have been flooded with distilled water for a prolonged period are compared with those that have been flooded for the same length of time with Hoagland solution.

Species	Ratio N ₂ : Air	
	Flooded in distilled water	Flooded in Hoagland solution
<i>Pinus contorta</i>	1.20	1.33
<i>Alnus incana</i>	1.14*	1.13*
<i>Larix laricina</i>	1.19	1.19
<i>Picea mariana</i>	1.23	1.94
<i>Picea sitchensis</i>	1.23	1.53†
<i>Pinus sylvestris</i>	1.14	1.49

*Alnus incana: mean of sample from "upper" and "lower"
(at least 17 cms below the soil surface) roots.

† Picea sitchensis: mean of two samples.

Table 3.10. The effect of anaerobiosis on the labelling of aspartic and glutamic acids as seen in the ratio nitrogen:air of the percentage label found in root extracts after a one hour fixation period in the relevant atmosphere. Unflooded tree seedlings are compared with those that have been flooded for a prolonged period with Hoagland solution.

Species	Ratio N ₂ : Air	
	Unflooded controls	Flooded in Hoagland solution
<i>Pinus contorta</i>	0.70	0.61
<i>Alnus incana</i>	0.54*	0.26*
<i>Larix laricina</i>	1.24	0.54
<i>Picea mariana</i>	0.40	0.55
<i>Picea sitchensis</i>	0.36	0.43†
<i>Pinus sylvestris</i>	0.38	0.54
<i>Larix europea</i>	0.55	0.68†

**Alnus incana*: mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

†*Picea sitchensis* and *Larix europea*: mean of two samples.

This is the same finding, as described above, in the comparison of unflooded seedlings with those which have been flooded for a prolonged period with distilled water (p.52). This complex problem of amino acid labelling appears to be linked to the balance of the various reactions affecting their pool size in the root cells. On the one hand, increased dark fixation into the amino acids in flood-tolerant species, flooded in Hoagland solution, produces an increase in the amino acid radio-activity. On the other hand, deproteinisation, particularly in flood-intolerant species, and in all plants to a certain extent when kept in distilled water, produces the reverse effect. (For a fuller account see Discussion P.88).

It is important to note that only in flood-tolerant species does the sum of labelled alanine, tyrosine, phenylalanine, glutamine and citrulline become less dependent on oxygen availability during a fixation period, after a pretreatment with Hoagland solution (Table 3.11). The reverse relationship to oxygen availability was found for the flood-intolerant species. In other words, in flood-intolerant species the labelling of the amino acids, other than aspartic and glutamic acid, is less enhanced under nitrogen than under air, but in flood-tolerant species, the sum of labelled alanine, tyrosine, phenylalanine, glutamine and citrulline is either more, or almost as much enhanced under nitrogen as under air.

In order to find further evidence for the possible explanation, given above, that in flood-tolerant species the addition of nutrients has an activating effect on the turnover of the amino acids, the distribution of some metabolites under nitrogen after a pretreatment with Hoagland

Table 3.11. The effect of anaerobiosis on the labelling of amino acids excluding the aspartic and glutamic acids as seen in the ratio Nitrogen:Air of the percentage label found in root extracts after a one hour fixation period in the relevant atmosphere. Unflooded tree seedlings are compared with those that have been flooded for a prolonged period with Hoagland solution.

Species	Ratio N ₂ : Air	
	Unflooded controls	Flooded in Hoagland solution
<i>Pinus contorta</i>	0.58	0.94
<i>Alnus incana</i>	0.54*	1.21*
<i>Larix laricina</i>	0.76	1.04
<i>Picea mariana</i>	0.74	-
<i>Picea sitchensis</i>	1.47	0.47†
<i>Pinus sylvestris</i>	1.08	0.43
<i>Larix europea</i>	0.79	0.69†

- No value under nitrogen

* *Alnus incana*: mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

† *Picea sitchensis* and *Larix europea*: mean of two samples.

solution and distilled water was compared (Fig. 3.1, Comparison 8). Table 3.12 reveals that the decreasing sequence of flooding-tolerance accompanies a decreasing ratio of the sum of the labelled amino acids, "flooded Hoagland solution: flooded distilled water" under nitrogen. In other words, additional nutrients during the pretreatment enhance the labelling of amino acids under nitrogen much more in the flood-tolerant species than in the flood-intolerant plants.

In Hoagland solution, there is a marked increase in the sum of labelled amino acids spots. This sequence of the sum of the labelled amino acids, which is always related to flood-tolerance, is not true for every individual amino acid, however, for the labelling of glutamic acid decreases in Pinus sylvestris, and alanine is not labelled after a prolonged flooding period with Hoagland solution in Picea mariana. Also, aspartic acid does not reveal a sequence, even if it increases in all species used (Table 3.13). However, a further possibility which may be related to the flood-tolerance of the seedlings can be found in the comparison of the sum of the labelled glutamic acid, alanine and citrulline activity, when the tree seedlings have been flooded for a prolonged period, some with distilled water and others with Hoagland solution (Table 3.14). In the flood-tolerant species, the relative labelling of these amino acids is more than doubled after a flooding period with Hoagland solution, when we compare it to that in distilled water, while in the flood-intolerant species, only a slight increase could be observed in the same comparison.

Table 3.12. Percentages of the sum of labelled amino acids after a $C^{14}O_2$ -fixation of roots for one hour under nitrogen when tree seedlings have been flooded for a prolonged period with distilled water and Hoagland solution.

Species	Flooded in distilled water (1)	Flooded in Hoagland soln. (2)	Ratio (2) : (1)
<i>Pinus contorta</i>	9.51	26.25	2.76
<i>Alnus incana</i>	8.65*	20.13*	2.33
<i>Larix laricina</i>	7.00	13.17	1.88
<i>Picea mariana</i>	14.21	20.24	1.42
<i>Picea sitchensis</i>	14.94	20.54†	1.38
<i>Pinus sylvestris</i>	15.11	19.52	1.29

**Alnus incana*: mean of sample from upper and lower (at least 17 cms under soil surface) roots.

† *Picea sitchensis*: mean of two samples.

Table 3.13. Percentages of labelled aspartic acid after $C^{14}O_2$ -fixation of roots for one hour under nitrogen when the tree seedlings have been flooded for a prolonged period with Hoagland solution, compared with distilled water.

Species	Flooded in Distilled water (1)	Flooded in Hoagland soln. (2)	Ratio (2) : (1)
<i>Pinus contorta</i>	1.43	5.44	3.80
<i>Alnus incana</i>	0.27*	2.97*	11.00
<i>Larix laricina</i>	2.17	2.90	1.34
<i>Picea mariana</i>	2.14	2.36	1.10
<i>Picea sitchensis</i>	3.82	7.28†	1.91
<i>Pinus sylvestris</i>	4.22	7.14	1.69

**Alnus incana*: mean of sample from "upper" and "lower" (at least 17cms below the soil surface) roots

† *Picea sitchensis*: mean of two samples.

Table 3.14. Percentages of the sum of labelled glutamic acid, alanine and citrulline after a $C^{14}O_2$ -fixation of roots for one hour under nitrogen when the tree seedlings have been flooded for a prolonged period with distilled water as compared with Hoagland solution.

Species	Flooded in Distilled water (1)	Flooded in Hoagland soln. (2)	Ratio (2) : (1)
<i>Pinus contorta</i>	8.08	19.82	2.45
<i>Alnus incana</i>	8.39*	17.98*	2.14
<i>Larix laricina</i>	4.83	10.27	2.13
<i>Picea mariana</i>	12.07	17.88	1.48
<i>Picea sitchensis</i>	11.12	13.26†	1.19
<i>Pinus sylvestris</i>	10.89	11.47	1.05

**Alnus incana* : mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

† *Picea sitchensis* : mean of two samples.

This might be taken as evidence that through additional nutrients, and mainly in the flood-tolerant species, the nitrification of organic acids is enhanced (see Discussion p.87).

In this context, it is noteworthy that the comparison of seedlings which have been flooded for a prolonged period with Hoagland solution and distilled water, and both incubated with $C^{14}O_2$, under air (Fig. 3.1, Comparison 10), shows that the sum of labelled tyrosine, phenylalanine, glutamine and citrulline increases in response to a pretreatment with flooding with additional nutrients. (Table 3.15).

In this connection, it was also found of interest to compare the results with unflooded seedlings with those from seedlings which have been pretreated with Hoagland solution (cf. Fig. 3.1, Comparison 11). The percentages of the sum of all labelled amino acids increase in all species used under nitrogen through a pretreatment with Hoagland solution, in contrast to what happens with the unflooded control plants. In particular a marked increase in aspartic acid is noted. (Table 3.16). Also the percentages of fumaric acid increase after a pretreatment with Hoagland solution, and this might be regarded as a consequence of an enhanced turnover rate of the transamination reactions (Table 3.17).

It may be further noted that under nitrogen, the labelling of citrulline is suppressed only in flood-intolerant species, the seedlings of which have been flooded

Table 3.15. Percentages of the sum of labelled tyrosine, phenylalanine, glutamine and citrulline after $C^{14}O_2$ -fixation of roots for one hour under air when the tree seedlings have been flooded for a prolonged period with Hoagland solution, and distilled water.

Species	Flooded in Distilled water (1)	Flooded in Hoagland solution (2)	Ratio (2) : (1)
<i>Pinus contorta</i>	-	6.21	-
<i>Alnus incana</i>	7.49*	11.26*	1.50
<i>Larix laricina</i>	0.90	3.12	3.47
<i>Picea mariana</i>	2.25	3.55	1.58
<i>Picea sitchensis</i>	3.54	9.86†	2.79
<i>Pinus sylvestris</i>	4.39	19.47	4.44

**Alnus incana* : mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

†*Picea sitchensis*: mean of two samples.

Table 3.16. Percentages of labelled aspartic acid after a $C^{14}O_2$ -fixation of roots for one hour under nitrogen when the tree seedlings have been flooded for a prolonged period with Hoagland solution compared with unflooded control plants.

Species	Unflooded controls	Flooded in Hoagland soln.	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	2.22	5.44	2.45
<i>Alnus incana</i>	1.01*	2.97*	2.94
<i>Larix laricina</i>	2.57	2.90	1.13
<i>Picea mariana</i>	1.52	2.36	1.55
<i>Picea sitchensis</i>	1.50	7.28†	4.85
<i>Pinus sylvestris</i>	2.54	7.36	2.90
<i>Larix europea</i>	1.48	4.42†	2.99

**Alnus incana* : mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

† *Picea sitchensis* and *Larix europea* : mean of two samples.

for a prolonged period with Hoagland solution. But in the flood-tolerant species Alnus incana, citrulline becomes more highly labelled in the roots after a similar flooding. Thus it seems that if in flood-tolerant species, additional $C^{14}O_2$ -fixation into citrulline occurs, then it becomes enhanced after a treatment with Hoagland solution. Yet in flood-intolerant species, this additional $C^{14}O_2$ -fixation becomes inhibited through a long flooding period with Hoagland solution, as has been found for seedlings flooded for long periods with distilled water (cf. p.49).

Not only does the incorporation of C^{14} into the amino acids increase after a flooding period with Hoagland solution, as compared to the unflooded control plants, but also the percentages of labelled phosphate esters and sugars are highly enhanced (Table 3.18).

This seems to indicate that the general turnover of metabolites, deriving from a $C^{14}O_2$ -fixation in roots, is greatly improved through a pretreatment with Hoagland solution, when compared with the unflooded control plants.

So far, the results for seedlings which have been flooded with Hoagland solution, were examined in detail only when incubated under nitrogen. But under air, there are other comparisons which can be made (cf. Fig. 3.1, Comparisons 10, 12). When seedlings have been flooded for a prolonged period with Hoagland solution, the sum of the percentages of labelled organic acids decreases and that of the amino acids increases (Comparison 12, 10). However, under air, the labelling of phosphate esters does not reveal

Table 3.17. Percentages of labelled fumaric acid after $C^{14}O_2$ -fixation of roots for one hour under nitrogen when the tree seedlings have been flooded for a prolonged period with Hoagland solution, compared with unflooded control plants.

Species	Unflooded controls	Flooded in Hoagland soln.	Ratio Flooded:unflooded
<i>Pinus contorta</i>	-	1.23	+
<i>Alnus incana</i>	1.08*	1.40*	1.30
<i>Larix laricina</i>	1.96	3.78	1.93
<i>Picea mariana</i>	0.85	0.98	1.15
<i>Picea sitchensis</i>	1.63	3.04†	1.87
<i>Pinus sylvestris</i>	1.84	3.49	1.90
<i>Larix europea</i>	0.69	2.32†	3.36

**Alnus incana* : mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

†*Picea sitchensis* and *Larix europea* : mean of two samples.

Table 3.18. Percentages of the sum of labelled phosphate esters and sugars after $C^{14}O_2$ -fixation of roots for one hour under nitrogen when the tree seedlings have been flooded for a prolonged period with Hoagland solution compared with unflooded control plants.

Species	Unflooded controls	Flooded in Hoagland soln.	Ratio Flooded:Unflooded
<i>Pinus contorta</i>	-	2.31	-
<i>Alnus incana</i>	1.10*	7.16*	6.51
<i>Larix laricina</i>	-	-	-
<i>Picea mariana</i>	-	11.79	-
<i>Picea sitchensis</i>	-	1.55†	-
<i>Pinus sylvestris</i>	-	1.55	-
<i>Larix europea</i>	-	-†	-

**Alnus incana* : mean of sample from "upper" and "lower" (at least 17 cms below the soil surface) roots.

†*Picea sitchensis* and *Larix europea* : mean of two samples.

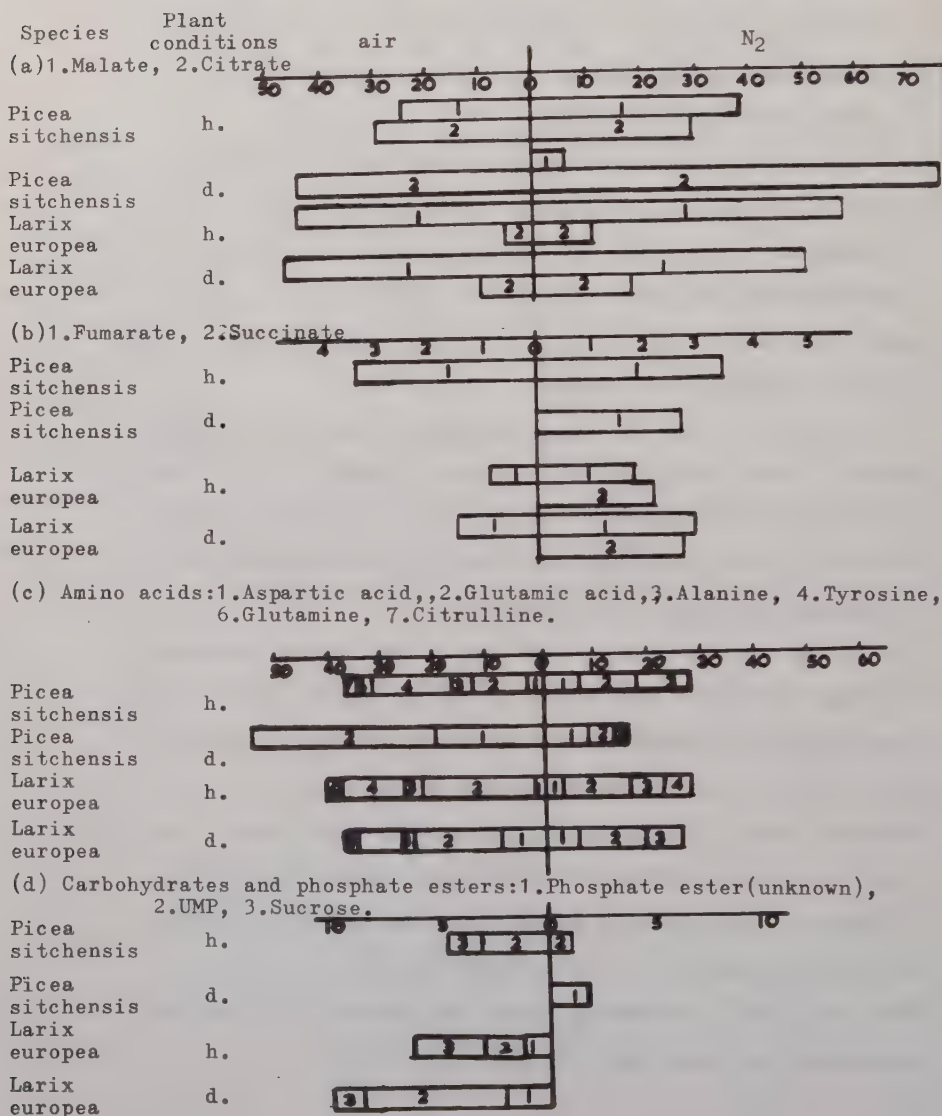
any regularity in the species used; nor is there any relationship apparent between the labelling of any metabolites and the flooding-tolerance of the species, when seedlings pretreated with Hoagland solution are compared with unflooded seedlings or with those which have been flooded with distilled water.

Thus it is necessary to point out that an incubation of $C^{14}O_2$ under air , is a very artificial experimental condition. But nonetheless it has helped to elucidate the regulation of the $C^{14}O_2$ metabolism in roots in relation to anaerobis, and the general flooding-tolerance of the species used, as demonstrated above.

All the values for Picea sitchensis and Larix europea, given in this paragraph 3.3, are from samples taken at random from trees suffering different amounts of flooding damage. However, in Fig. 3.15 the analyses for flood-damaged and undamaged trees are shown separately.

Under nitrogen, the labelling of malic acid decreases, and that of citric acid increases in the roots of the damaged seedlings, when compared with those of the undamaged plants. The total for labelled amino acids decreases under N_2 in the damaged plants, yet aspartic acid becomes more highly labelled. Under air, the labelling of citric and aspartic acid also increases in the damaged plants, but the number of labelled amino acids and their level of radioactivity decreases. That is to say, in damaged plants the labelling of alanine, glutamine, tyrosine and citrulline decreases.

Fig. 3.15. Percentages of total radioactivity incorporated into the organic acids, amino acids, carbohydrates and phosphate esters after a $C^{14}O_2$ -fixation for one hour by roots of Picea sitchensis and Larix europea seedlings incubated under air, and then under N_2 . The seedlings have been flooded for a prolonged period with Hoagland solution. Each value is a mean of three measurements.



h = healthy

d = damaged

Finally, the values of Alnus incana, given above, are means for the "lower" (at least 17 cms under the soil surface) and "upper" roots. Fig. 3.5 shows that in seedlings which have been flooded with Hoagland solution for a prolonged period under nitrogen, the labelling of the fumaric and succinic acids is reduced, and furthermore, that under air and nitrogen, the number of labelled carbohydrates and phosphate esters decreases in the "lower" roots. However, the sum of the percentages of labelled amino acids increases not only under nitrogen, but also under air in the "lower" roots. Yet, under air, the number of labelled amino acids is also reduced.

This reveals not only that the roots of different species yield a distinctive pattern in some of their labelled metabolites after a $C^{14}O_2$ -fixation in relation to their flooding-tolerance, but also that it is of importance to collect a representative root-sample for any comparison between species. In other words, different root layers may reveal different distribution patterns in their labelled metabolites, as found above with 3-4 year old Alnus incana seedlings. Also, any variation in the appearance of the shoot, due to a prolonged pretreatment, has to be taken into account in any comparison between different species, in relation to the general flooding-tolerance.

Conclusion

1. The distribution pattern of labelled metabolites seems to reveal that the enhanced $C^{14}O_2$ -fixation under air (cf. Chapter II, p.31) is accompanied by a relatively higher

labelling in those compounds which are dependent on a sufficient supply of NADH and ATP for their synthesis. Hence, under anaerobic conditions, the transamination reactions may be more important in accounting for the turnover of the labelled metabolites.

2. It seems likely that the distribution of labelled metabolites after a pretreatment with distilled water indicates that in the flood-tolerant species under anaerobic conditions, the deproteinisation processes slow down, in contrast with the unflooded control plants. Yet, under anaerobic conditions, the nitrification processes seem to be more enhanced in the flood tolerant species, after a pretreatment with Hoagland solution, again in contrast to the flood-intolerant species.

3. It is of particular interest that some sequences in the labelling of metabolites were detected which are related to the classification of flooding-tolerance among the species used, as described in the previous Chapters. Thus, further classification tests for the flooding-tolerance of tree seedlings became apparent.

(i) The dependence of the total amount of labelled malic acid on oxygen availability decreases with increasing flooding-tolerance of the species used. This test is always valid for trees that have had no flooding pretreatment.

(ii) Under nitrogen, the percentages of labelled glutamic acid decrease in the roots of flood-tolerant species, after a prolonged pretreatment with distilled water, but increases with the decreased flood-tolerance of the species used. Thus when pretreated and unflooded control plants are compared, the labelling of glutamic acid

is inversely proportional proportional to the flooding-tolerance of the species.

(iii) Under nitrogen, additional nutrients during a prolonged pretreatment enhance the percentages of labelled amino acids with increasing flooding-tolerance of the tree seedlings.

(iv) A sequence for flooding-tolerance is also obtained when the sum of the percentages of labelled glutamic acid, alanine and citrulline is compared. Here, the increase in the flooded over the unflooded plants is directly proportional to the flooding-tolerance of the species used. (Trees in this test are flooded with Hoagland solution).

4. Furthermore, in all flood-tolerant species, certain characteristics were found to be valid, distinguishing them from the flood-intolerant seedlings:

(i) A four day pretreatment with distilled water and under nitrogen increases the sum of percentages of labelled amino acids and, in particular, aspartic acid.

(ii) Under nitrogen, the percentages of labelled citric acid increase only in flood-tolerant species, due to a prolonged pretreatment with distilled water. The reverse was found for the percentages of the labelled amino acids, or, the individual percentages of alanine, aspartic and glutamic acids.

(iii) However, under air, the percentages of labelled tyrosine decrease after the flood-tolerant seedlings have been pretreated for a prolonged period with distilled water.

(iv) Furthermore, the percentages of labelled aspartic and glutamic acid are less affected by changes in the oxygen

supply during the $C^{14}O_2$ -fixation of the roots, after a prolonged pretreatment with distilled water.

(v) The percentages of labelled organic acids are almost as much affected by changes in the oxygen supply during the $C^{14}O_2$ -fixation of the roots, after a prolonged pretreatment with distilled water, as with Hoagland solution.

(vi) The comparison of the percentages of the labelled aspartic and glutamic acids show an enhanced dependence on oxygen supply, when the tree seedlings have been flooded for a prolonged period with Hoagland solution.

Yet when the data of the same experiments are compared, the sum of the percentages of labelled alanine, tyrosine, phenylalanine, glutamine and citrulline reveal the converse pattern.

(vii) If citrulline is labelled under nitrogen, its percentages increase after prolonged pretreatment with Hoagland solution.

5. With all species used, certain regularities in some metabolites can be observed, which appear to be linked to the various experimental conditions, and are not related to the flooding tolerance of the trees.

(i) In unflooded tree seedlings, sucrose is always included whenever carbohydrates become labelled, and the labelling of phosphate esters decreases under nitrogen as compared with air. Tyrosine and glutamine become labelled under air, and the labelling of glutamic acid increases under air, while the labelling of alanine is higher under nitrogen. Malic acid is always the most highly labelled product of the $C^{14}O_2$ -fixation; succinic and fumaric acids are ten times less labelled than the malic and citric acids

together.

(ii) A comparison between seedlings which have been flooded for four days with distilled water, and the unflooded controls, reveals that under nitrogen, the percentages of labelled malic acid increase, while those of the citric and succinic acids decrease.

(iii) When we compare tree seedlings which have been flooded for a prolonged period with distilled water, and the unflooded control plants, we see that the sum of the percentages of labelled organic acids is higher under nitrogen than under air, while the sum of percentages of labelled amino acids, carbohydrates, and phosphate esters is higher under air than under nitrogen.

(iv) Unflooded tree seedlings compared with those that have been flooded for a prolonged period with Hoagland solution, show that under nitrogen and air, additional nutrients cause a decrease in the sum of the percentages of labelled organic acids, but that the sum of the percentages of labelled amino acids, and in particular aspartic acid, increases. The labelling of fumaric acid, carbohydrates and phosphate esters is only increased under nitrogen.

(v) If seedlings which have been flooded for a prolonged period with distilled water and Hoagland solution are compared, it becomes apparent that under nitrogen and air, the sum of the percentages of labelled organic acids decreases. Yet, under air and nitrogen, the sum of the percentages of amino acids increases; under nitrogen the percentages of labelled aspartic acid increase, and under air the sum of the percentages of labelled tyrosine, phenylalanine, glutamine and citrulline rise.

69.

6. Finally, it is important to note that the root samples collected for the experiments have to be representative for the species used, since different root layers and any variation in the appearance of the shoots seem to result in a different distribution pattern of the labelled metabolites, deriving from the $C^{14}O_2$ -fixation of the roots.

CHAPTER IV.

Quantitative Changes in Certain Tree Root Metabolites in Relation to Flooding Tolerance.

In Chapter III it had become apparent that, apart from the differences in the distribution pattern of metabolites after $C^{14}O_2$ -fixation in the roots of flood-tolerant and intolerant tree seedlings, there are also certain distinctive characteristics concerning the total amounts present of certain substances that are binding for all species used. The number of studies published concerning the tolerance of anoxia by trees is small, presumably due to the specialised nature of such investigation. However, anaerobic conditions within trees were reported as early as 1899 by Devaux, who studied the respiratory rates of detached branches and also detected the presence of ethanol in wood-distillate. Čirková (1968) reported a higher accumulation of lactic acid, a typical product of anaerobic respiration, in the roots of willow (Salix alba L.) than in poplar seedlings (Populus petrowskiana Schroed.). Hook et al. (1970) have reported an increased production of ethanol in incubated roots of swamp tupelo seedlings (Nyssa sylvatica). It was therefore thought desirable to determine changes in the most abundant carbohydrates in roots as well as some anaerobic respiration products in relation to waterlogged soil conditions.

1. Changes in the fructose, glucose and sucrose content of roots due to experimental flooding.

As many of the carbohydrates found in plants continuously

undergo conversions from one form to another, or are being transformed into compounds used in respiration or the synthesis of fats, proteins or other non-carbohydrates, it seemed justifiable that an investigation be made of the changes in the amount of some carbohydrates of roots in relation to experimental flooding. Fig. 4.1 shows the results of some preliminary experiments in which the principal sugars present were estimated by gas-liquid chromatography. The results reveal that the amounts of fructose and glucose alone decrease in the flood-intolerant species Larix europea during a short period of experimental flooding (0 - 6 days) and not in Picea sitchensis, whose changes in these carbohydrates are more similar to those of the flood-tolerant species Pinus contorta. However, in the flood-intolerant species the amount of sucrose is higher than in the two provenances of Pinus contorta seedlings, examined after the seedlings have been flooded for 2 and 8 days. Furthermore, a comparison of the total amount of fructose, glucose and sucrose of roots (0, 2, 4, 6 and 8 days flooded) shows that the sum of sucrose is higher in Picea sitchensis than in Larix europea and the two provenances of Pinus contorta. In addition, it is of note that in the more flood-tolerant group of Pinus contorta examined, the total amount of these three carbohydrates is higher than in the less flood-tolerant seedlings. These findings would suggest in total that carbohydrate metabolism is altered rapidly by flooding treatments but the behaviour of each individual sugar varies widely from species to species. Thus further experiments are needed to solve the problem of whether these differences are related to flooding-tolerance of tree seedlings or not.

Fig. 4.1. Changes in the fructose, glucose and sucrose content of roots due to experimental flooding.

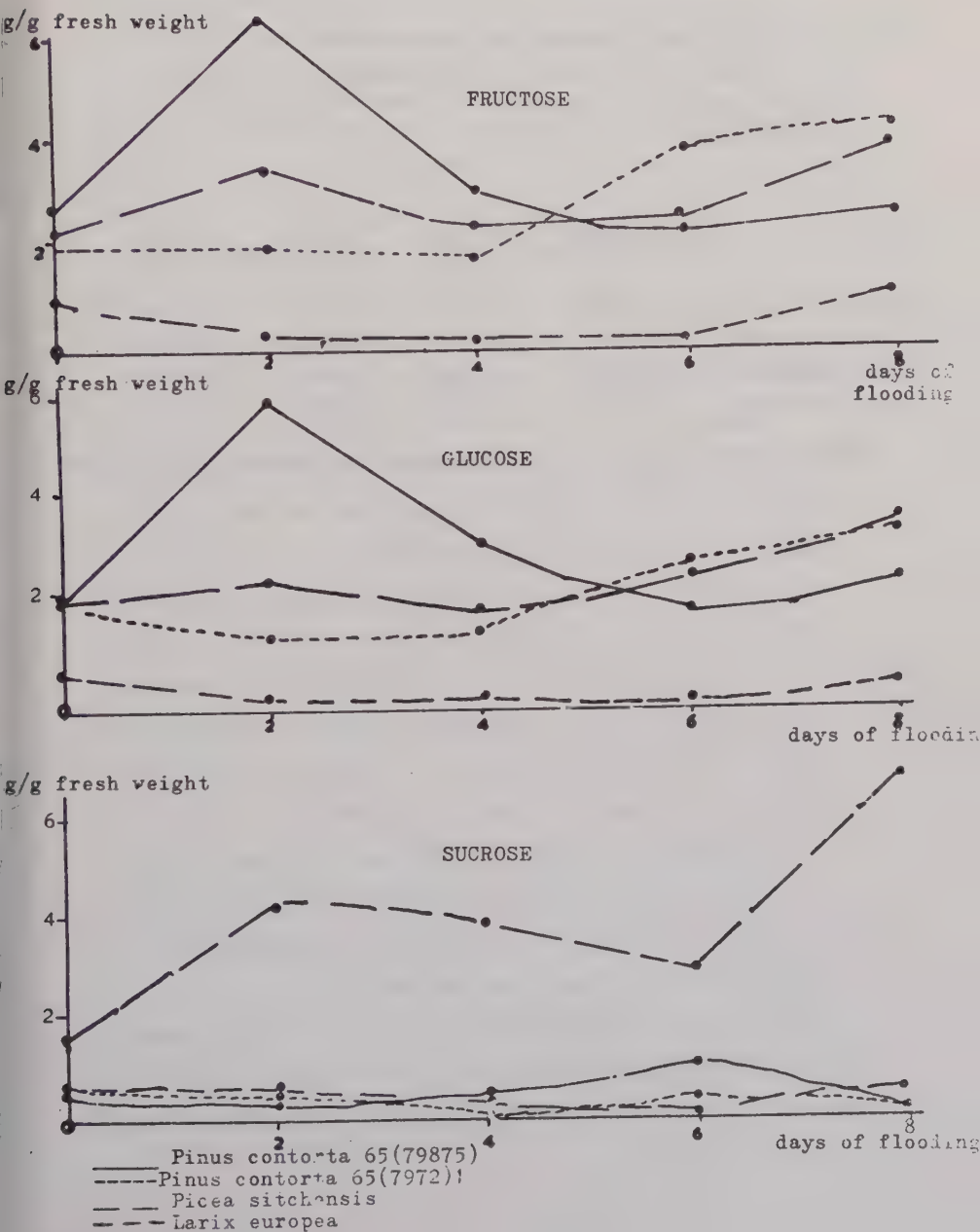


Table 4.1. Fructose, glucose and sucrose contents in roots of tree seedlings that have been flooded for 0, 2, 4, 6 and 8 days. Each value is the mean of five measurements.

Species	Provenance*	Fructose	Glucose	Sucrose
		µg/g fresh weight of roots		
Pinus contorta	67(7987)5	3.41±.77	3.18±.73	0.54±.16
Pinus contorta	65(7972)1	2.87±.56	2.31±.40	0.40±.12
Picea sitchensis	66(7111)1	2.98±.31	2.60±.30	3.92±.83
Larix europea	62(4371)	0.59±.19	0.39±.13	0.49±.11

*Numbers refer to the Forestry Commission's provenances register.

2. Ethanol and Malic Acid content of Birch Sap in relation to dry and wet habitats.

Regarding a number of non flood-tolerant herberous plants (Crawford, 1966, 1967) it has been shown that flooding caused an increase in the rate of glycolysis leading to an increased production of ethanol. Yet, in a study of the metabolism of roots of plants native to British bogs, Crawford and Tyler (1969) noted that those species which are able to withstand flooding increase the malic acid content of their roots when they are flooded.

Therefore it was thought to be of interest to examine the rising sap taken from mature birch trees (Betula pubescens) in early April. Photograph B shows how the sap was collected and Table 4.2 records the ethanol and malic acid content of sap expressed in μM per litre. The very small ethanol content increases in the sap of those trees from a wet site compared with those from a dry site. As the detection of ethanol in plant tissues can usually be considered as a symptom of oxygen deficiency, these results suggest that in mature trees such as birch, the roots are subjected to anaerobic conditions, at least for a certain period of the year, and that this is not prevented by a supply of oxygen from the aerial portions of the plant. Yet, the important discovery is that the malate concentration is found to be approximately 100 times greater than that of ethanol, and in wet sites the malic acid content was twice as high as in dry sites. Mazelis and Vennesland (1957) had suggested that on theoretical grounds of enzyme equilibria,

Table 4.2. A comparison of the ethanol and malic acid contents of sap from birch trees (Betula pubescens) growing in wet and dry soils. The samples are taken in early April before bud break and each figure is the mean of three samples.

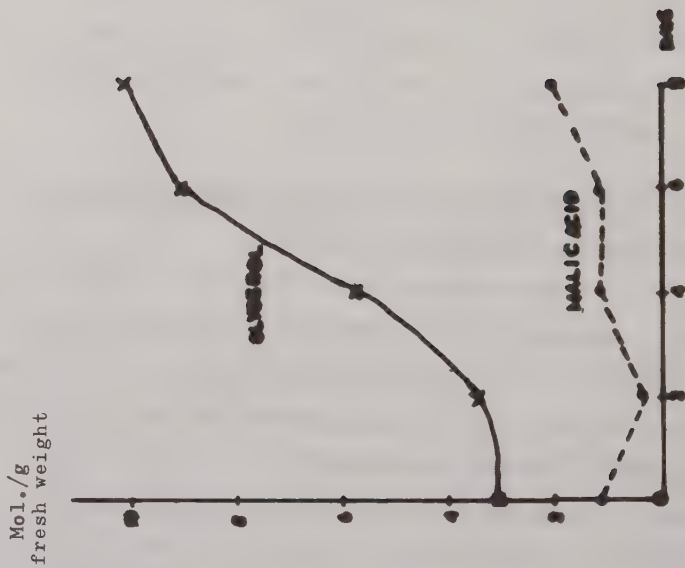
Soil type	Water status of soil	Ethanol $\mu\text{M/litre}$ of sap	Malic acid $\mu\text{M/litre}$ of sap
Peat	Wet	13.5 ± 0.75	3850 ± 340
Peat	Dry	9.9 ± 2.09	1870 ± 330
Sand	Wet	31.9 ± 9.4	3170 ± 130
Sand	Dry	13.4 ± 4.5	2490 ± 360

malate could be the natural anaerobic end product of respiration in plants. It thus appears likely that when these roots of Betula pubescens respire anaerobically, they produce large quantities of malate, which is translocated upwards in the rising sap. The malic acid would then provide useful carbon skeletons for further synthetic processes in leaves, so that the end result would be a net gain of carbon to the plants, derived from carbon dioxide fixation of the roots.

3. Glycerol and Malic Acid content of *Alnus incana* roots in relation to experimental flooding.

There is increasing evidence that higher plants produce a greater range of anaerobic respiration products than the above mentioned selection of lactate, ethanol and malate. In alder roots (*Alnus incana*), it was found that the glycerol content rises markedly in 3 - 4 year-old seedlings during the first few days of experimental flooding with distilled water (Fig. 4.2). Glycerol is a non-toxic end product which can accumulate without injury to the plant. In addition, glycerol is also a substance which is likely to increase the internal osmotic pressure of the cells without any significant increase in the intracellular ionic concentration. Further, if one starts the pathway leading to glycerol with one molecule fructose 1,6-diphosphate, and postulates that with one molecule of glycerol one molecule phosphoenol pyruvate is formed as well, then recycling of NAD is possible. A net gain in ATP will also be made in the production of glycerol from glycerophosphate.

Fig. 4.2. Changes in free glycerol and malate content of Alnus incana roots in relation to flooding.



Yet, glycerol is not a compound of the Krebs cycle, and therefore it might not be so easily further metabolised, as, for instance, malate. Therefore it is important to note that preliminary experiments revealed that the content of malic acids increases also in roots after an experimental flooding period of 8 days (cf. Graph 4.2).

4. Internal Gases in Shoot and Root of tree seedlings in relation to flooding tolerance.

Some attention has already been given by other authors to the internal plant atmosphere, and this is summarized by Hartmann and Brown (1967). Methane has been detected as a constituent of the internal atmosphere of some submerged vascular aquatic plants growing in ponds with organic substrates; and it had been suggested that methane diffuses from the anoxic substrate into the roots or rhizoidal portion of the plants (Hartmann, Brown, 1966). It is known that in permanently waterlogged anaerobic soils, Clostridium species may convert organic matter into organic acids, carbon dioxide and hydrogen, which are in turn converted by certain strictly anaerobic bacteria to methane (Hawker and Linton, 1971). Vlamis and Davis (1944) found that methane inhibits the growth of barley, but enhances that of rice. Therefore it was thought worth while to examine the concentration of methane in tree seedlings, after flooding.

Hartmann and Brown (1966) pointed out that the concentration of methane in submerged vascular aquatic plants is positively associated with the photosynthetic activity of the plants.

In this present study, both the roots and shoots of each plant were examined in order to obtain a comparable shoot:root ratio for each sample. Table 4.3 summarizes the results obtained for several flood-tolerant and intolerant tree seedlings of differing age and flooded with distilled water for varying periods. It is apparent from Table 4.3 that not only does the methane content of roots depend on the duration of waterlogged soil conditions but that it is also dependent on the age of the seedlings and species examined. Also, a much higher internal methane flow seems to occur from the roots to the shoot in the flood-tolerant seedlings as compared with the non-tolerant species. Even the more flood-tolerant provenance of Pinus contorta (67(7987)5) reveals a higher shoot:root ratio for methane content than the less tolerant group (65(7114)1). It thus seems likely that the diffusion of methane into the roots, and from there into the aerial portions of flood-tolerant tree seedlings, is a significant pathway for release of methane from the soil, and is much more pronounced in flood-tolerant species. It is interesting to note that when the soil is submerged with Hoagland solution the methane content of the roots and shoots is less in all cases (Table 4.4). It is of interest to compare the concentration of methane in Picea mariana by itself. The seedlings of this species not only grew better when they had been flooded with Hoagland solution, but also revealed with this treatment a higher shoot:root ratio of methane content as compared to the treatment with distilled water. Finally, it should be pointed out that only on a warm, sunny day and not on

Table 4.3. Distribution of methane in roots and shoots of tree seedlings when they have been flooded for (a) four weeks, (b) nine months and (c) 14 months with distilled water. With two- and three-year-old seedlings gas samples were obtained using the entire shoot or root system, but with older tree seedlings only the lowest side branches and roots were examined. The concentration of methane is given in μl per 1 ml of internal gas.

Species	Provenances*	Age in years	METHANE		Ratio
			root	shoot	shoot/root
(a) 4 weeks flooded					
Pinus contorta	65(7951)1H	2	757	216	0.29
Pinus contorta	65(7951)5	2	203	135	0.67
Picea sitchensis	66(711)1(3)	4	35,135	135	0.004
Pinus sylvestris	64(4122)11	4	5,405	162	0.03
(b) 9 months flooded					
Pinus contorta	65(7951)1H	2	116,216	114,865	0.99
Pinus contorta	65(7951)5	2	1,554	1,000	0.64
Picea mariana	69(718)	3	8,108	2,054	0.25
Picea sitchensis	66(711)1(3)	3	163,374	1,568	0.01
Pinus contorta	65(7987)5	7	34,460	9,730	0.28
Pinus contorta	65(7114)1	7	10,460	2,230	0.21
Larix europea	62(4371)	7	4121,622	1,041	0.0003
(c) 14 months flooded					
Alnus incana	66(4127)500	7	141,892	2,757	0.02

*Numbers refer to the Forestry Commission's provenance register.

Table 4.4. Distribution of methane in root and shoot of tree seedlings when they have been flooded for nine months with Hoagland solution. With two and three year-old seedlings gas samples were taken from the entire shoot and root system, but with seven year-old tree seedlings only the lowest side branches and roots were examined. The concentration of methane is given in $\mu\text{l/ml}$ of internal gas.

Species	Provenances*	Age in years	METHANE		Ratio shoot:root
			root	shoot	
Pinus contorta	65(7951)1H	2	1,451	387	0.27
Pinus contorta	65(7951)5	2	1,541	441	0.29
Picea mariana	69(718)	3	1,081	738	0.68
Pinus contorta	67(7987)5	7	3,041	576	0.19
Pinus contorta	65(7114)1	7	2,657	432	0.16

cold, dark days (with only artificial light in the greenhouse) traces of ethylene and acetylene could also be detected in addition to methane (Table 4.5). However, only the two flood-intolerant species Picea sitchensis and Pinus sylvestris reveal a higher ethylene content in the roots than in the shoot. As ethylene is known to have an effect on the plant's growth, even in very low concentration (Osborne, 1968), its occurrence on warm days might be of some significance for plants growing in waterlogged soil (Smith and Russell, 1969).

Conclusion.

A quantitative study of the changes of some metabolites and organic gases in waterlogged soil conditions showed that:-

- 1) The distribution pattern of methane seems to indicate a higher methane flow from the roots to the shoot in the flood-tolerant tree seedlings than in the non-tolerant species during experimental flooding.
- 2) Glycerol, an anaerobic respiration product, was found to increase during the first few days of experimental flooding in Alnus incana roots, and malic acid increases after 8 days flooding in the roots of this flood-tolerant species.
- 3) In the rising sap of mature Betula pubescens trees, it was found that not only the malate concentration is always approximately 100 times greater than the ethanol content, but also that the malic acid content is twice as high in wet sites as in dry sites.

Table 4.5. Distribution of two internal gases on a warm, sunny day when the tree seedlings have been flooded for (a) four weeks, (b) nine months and (c) 14 months with distilled water. With two and three year-old seedlings the entire shoot or root system was used but with older tree seedlings only the lowest side branches and roots were examined. The concentration of the gases is given in $\mu\text{l}/\text{ml}$ of internal gas.

Species	Provenances*	Age in years	ETHYLENE		Ratio
			shoot	root	shoot:root
(a) Four weeks flooded					
Pinus contorta	65(7951)1H	2	200	150	0.75
Pinus contorta	65(7951)5	2	200	50	0.25
Picea sitchensis	66(711)1(3)	4	50	600	12.00
Pinus sylvestris	64(4122)11	4	1400	2600	1.86
(b) Nine months flooded					
Pinus contorta	65(7951)1H	2	1500	100	0.67
Pinus contorta	65(7114)1	7	2700	550	0.20
Larix europea	62(4371)	7	16000	200	0.01
(c) Fourteen months flooded					
Alnus incana	66(4127)500	7			
ACETYLENE					
(a) Four weeks flooded					
Pinus contorta	65(7951)1H	2	1500	330	0.22
Pinus contorta	65(7951)5	2	500	100	0.20
Picea sitchensis	66(711)1(3)	4	50	550	11.00
Pinus sylvestris	64(4122)11	4	1200	350	0.29
(b) Nine months flooded					
Pinus contorta	65(7951)1H	2	0	400	-
Pinus contorta	65(7114)1	7	0	0	-
Larix europea	62(4371)	7	0	350	-
(c) Fourteen months flooded					
Alnus incana	66(4127)500	7	400	0	-

*Number refers to the Forestry Commission's provenance register.

77.

4) A relation between the content of fructose, glucose and sucrose in the roots of tree seedlings and the sequence of flooding-tolerance of the species used could not be ascertained. Yet, the sucrose content of roots was found to be the highest in the non-tolerant species, Picea sitchensis, and the more flood-tolerant provenance of Pinus contorta reveals the highest total amount of the three carbohydrates examined.

5) Ethylene was detected in flooded tree seedlings only during warm and sunny days.

DISCUSSION

The essential object of this study was to carry out some basic preliminary investigations necessary in the quest for an eventual simple test of flooding tolerance in tree seedlings. From a practical viewpoint it would be advantageous to determine at an early stage whether mature trees of the same species and provenances will be resistant to the usually detrimental effects of flooding. To begin with, a selection of tree seedlings provided by the Forestry Commission had to be classified. This was done by means of a growth test, and this had already been successfully applied as a quick flooding -tolerance check for herbaceous plants (Crawford, 1966).

But in this present study with trees it was not found possible to distinguish tolerant from intolerant plants by observing the growth rate of seedlings during the first half-year of flooding. For instance, even the flood-tolerant species, Alnus incana, which one can find frequently on soils with a high water-table, reduces its growth in a waterlogged soil. However, when the water-table was lowered, further observation of the growth of the tree seedlings enabled a primary classification to be made for the species and seedlings of different provenances used. During the recovery period, some species resume growth, to almost the same rate as their unflooded controls, while others appear to have suffered permanent damage.

Further, the onset of dormancy in shoot growth during an experimental flooding period proved to be of help in distinguishing between those species and provenances which show resistances to flooding, and those which are severely

damaged in waterlogged conditions. In less tolerant species for example the onset of dormancy in shoot-growth was found to take place earlier.

Overall, the root:shoot ratio of Pinus contorta seedlings provided a reliable guide to the determination of the relative flood-tolerance in the different provenances. An increase in the natural tendency of a variety to produce roots is accompanied by an increased tolerance of flooding. This is not surprising, since waterlogged soil damages or kills roots, resulting in a restriction of the plant's supply of water and minerals (Kramer, 1960). Thus, where there is a higher root:shoot ratio, there does seem to be a greater capacity to withstand waterlogged conditions than in varieties with a lower root:shoot ratios.

It is generally supposed that the inability of most trees to survive in waterlogged conditions is due not so much to the water itself, as to anoxia. And as was already indicated in the Introduction, there are two possible adaptive mechanisms in plants known to be resistant to flooding which may enable them to maintain a living root-system in waterlogged soils, viz. (i) morphological adaptations facilitating gas-exchange between the root and shoot and (ii) metabolic properties.

Regarding the first, nearly all tree species used in plantations are total incapable of surviving in soils deficient in oxygen. Yet in contrast, trees on natural sites are markedly different. Why then do wild trees differ in this respect? Opinion is divided on the matter. Some

believe it is to be explained by the different rates of oxygen-diffusion from the shoot to the root. Undoubtedly this does take place, and it is an important factor in helping roots to survive periods of oxygen stress. The avoidance of anoxia, rather than the attempt to resist it, is one type of adaptation very common in plants. It can be seen in the shallow rooting-systems of many of the tree-species that grow in wet areas. One of the most notable examples of this adaptation is the black spruce Picea mariana which grow in the wet muskeg soils of northern Canada. During the course of this study, it was noted that some tree seedlings form roots on the soil surface in relation to flooding. In general, seedlings which form new roots visible on the soil surface were found to be more successful in withstanding the stress of a surplus water supply. However, following growth tests on flooded and unflooded tree seedlings of different ages and heights, (see p.21) it seems that an adequate supply of oxygen from the shoots to the roots is a characteristic more of young tree seedlings than of mature trees. In each instance, growth was hindered by flooding, but the important observation was that in flood-intolerant species the older and larger the seedling, the more prone it is to damage, while in the flood-tolerant species, the ability to resist damage seems to be independent of age and size. This consideration suggests that in mature trees which withstand fllooding, it is not so much the oxygen supply which is the crucial factor enabling survival, as some other process. However, there is a need for more

investigations into the oxygen flux to the roots via the shoots, particularly in trees, as most workers have only investigated this phenomenon in herbaceous plants (Armstrong, 1964, 1967; Greenwood, 1967, 1969).

In this present study an examination was made of the internal gases in tree seedlings. Here the distribution pattern of methane showed a clear distinction between flood-tolerant and non-tolerant species. Not only was it found that in soil submerged in Hoagland solution, the methane content of the shoots and the roots is less than when flooded with distilled water, but also that under experimental flooding conditions, a higher methane-flow from the roots to the shoots seems to take place in tree seedlings which are flood-tolerant. It thus would seem that the diffusion of methane into the roots, and from there into the aerial portions of the plant, is a significant pathway for the release of methane from the soil. Thus, if methane can diffuse more readily upwards it may be that oxygen can move more easily downwards. Thus, although gas diffusion properties vary in relation to flooding tolerance, the earlier experiment on the effect of size on this, suggests that in mature trees physical adaptations alone will not enable the plants to survive prolonged periods of anoxia.

When we come to investigate any of the metabolic processes, it is always very striking how few pathways account for the majority of metabolic functions, and how these pathways are common to most living organisms, bacteria, angiosperms and mammals. This would suggest that for any

metabolic situation there is an optimal solution. From this, it should follow that any metabolic solution to the problems created by a certain environmental stress should also be similar in all organisms able to withstand the stress, irrespective of their systematic relationship. In other words, metabolic adaptations are determined by necessity, and are common to all adapted organisms, and are not determined by the accidents of heredity. When a land animal dives under water, or the root system of a plant becomes submerged, one of the first metabolic changes common to them all is a reduction in the overall rate of metabolism. In those mammals which can remain submerged for prolonged periods, such as seals, beavers or sounding whales, the oxygen supply to the muscles is cut off during submersion, and these tissues respire anaerobically with the production of lactate (for a general account, vid. Bryant, 1971). With plants raised in Knop's solution, Čirková (1968) showed that not only is the willow (Salix alba L.) more resistant to anaerobic conditions than poplar (Populus petrowkiana Schroed.), but also that in willow roots, the amount of lactic acid is twice as much as in the poplar roots. In other words, a non-toxic anaerobic respiration product increases the ability to resist a period of anoxia.

Under anaerobic conditions, however, lactic acid is not the only product produced. In the course of experimenting with alder roots (Alnus incana) the glycerol content was found to rise rapidly during the first few

83.

days of flooding (p.73). It is known that glycerol is produced not only in yeast when poisoned with bi-sulfite, but also in diapausing silkworm eggs. And blood inhabiting African trypanosomes produce considerable quantities of glycerol as well (von Brand, 1966). But while the formation of glycerol still yields ATP and permits the anaerobic re-cycling of NAD-NADH, it will also avoid the production of potentially toxic quantities of ethanol. Thus it is possible for glycerol production to continue without limit, even under anaerobic conditions.

Further, in the study of the malic acid and ethanol contents in birch sap, the malate concentration was found to be circa 100 times greater than that of ethanol. It has already been established that malate is a non-toxic anaerobic respiration product in the roots of plants indigenous to British bogs (Crawford & Tyler, 1969), as well as in some parasites (von Brand, 1966). Hence, it cannot be completely ruled out that among roots in general, anaerobic respiration produces malic acid, and that the oxygen debt of the roots to the plants is repaid by translocating the malic acid upwards in the rising sap - for further use in aerobic conditions. Yet, while the preliminary investigation into the total amount of fructose, glucose and sucrose in roots revealed changes due to experimental flooding, further experiments will be necessary to ascertain if those differences are related to flooding-tolerance.

Apart from these considerations, however, there is growing evidence that, like animals, plants produce a

wider range of substances in adapting to anaerobic environmental conditions than the usual selection of ethanol and lactate. Glycerol and malate have now been noted (viz. Table C) and these discoveries suggest that there may well be additional substances produced under anaerobic conditions which might be of use in this present endeavour to establish an eventual simple classification test, based on the production of specific metabolites. Yet it is not just an investigation into anaerobic respiration products which may yield fruitful results, but also the hitherto little studied process of carbon dioxide fixation in roots. Only rarely have investigations been made into the non-photosynthetic $C^{14}O_2$ -fixation of roots in relation to stress brought about by periods of anoxia (Poel, 1953). In this study, using only tree seedlings, this method was used to establish if there are (i) quantitative changes in the total $C^{14}O_2$ -fixation and CO_2 -evolution of roots and (ii) qualitative and quantitative changes of single metabolites in various species, in respect to their differing flood-tolerances.

Regarding the first, it became apparent that a typical result of anoxia is that it hinders the incorporation of C^{14} into the general metabolism. In the quest for a simple classification test of the flooding-tolerance of tree seedlings, it is of greater importance to compare the rate of $C^{14}O_2$ -fixation under nitrogen and under air. It is this ratio that predicts accurately the degree of flood-tolerance in seedlings up to three years

Table C. Substances reported to accumulate under conditions of partial or complete anoxia in higher plants and animals. (References do not indicate priority of discovery and are intended only as a source of further information.)

Substance	Occurring in animals	Reference	Occurring in higher plants	Reference
Lactic acid	+	von Brand (1966)	+	Wager (1961)
Pyruvic acid	+	von Brand (1966)	+	Dubinina (1962)
Acetic acid	+	von Brand (1966)		
Propionic acid	+	von Brand (1966)		
Butyric acid	+	von Brand (1966)		
Succinic acid	+	von Brand (1966)	+	Wager (1962)
Glycerol	+	von Brand (1966)	+	Crawford (1972)
Ethyl alcohol	+	von Brand (1966)	+	Fulton & Erickson (1964)
Carbon dioxide	+	von Brand (1966)	+	James (1951)
Hydrogen	+	von Brand (1966)		
Shikimic acid			+	Henshaw <u>et al.</u> (1962)
Malic acid	+	von Brand (1966)	+	Crawford & Tyler (1969)
Glycolic acid			+	Dubinina (1962)
Glyoxalic acid			+	Dubinina (1962)
α ketoglutaric acid			+	Dubinina (1962)
Sorbitol	+	Gilmour (1965)		
Alanine	+	Gilmour (1965)	+	Dubinina (1962)
Ethylene			+	Pratt & Groeschl (1969)
γ amino butyric acid			+	Fulton <u>et al.</u> (1964)
Glutamic acid			+	Dubinina (1962)
Serine			+	Dubinina (1962)
Proline			+	Dubinina (1962)
α methyl butyrate	+	Bryant (1971)		
α methyl valerate	+	Bryant (1971)		
α glycerophosphate	+	Gilmour (1965)		

old. That is to say, in flood-tolerant species, the total $C^{14}O_2$ -fixation of roots is less dependent on oxygen than it is in flood-intolerant species.

As well as these findings, changes of the R.Q. were observed in the course of these experiments. The occurrence of a high R.Q. is indicative of persistent anaerobic respiration (Beevers, 1961). Hence it was of interest to observe that only in flood-intolerant species whose seedlings had been flooded for a long period was the R.Q. of the roots increased above one.

While the results obtained in this study from the investigation of the qualitative and quantitative changes of single metabolites in various tree species, in relation to their flooding-tolerance, were of interest, they are however so extensive that some simplification is needed to form a basis for a flooding-tolerance test. Poel (1953) drew attention to the fact that although the general pattern of dark-fixation is common to a wide range of organisms and tissues, the relative rates of the intermediate reactions are very variable. If, however, in unflooded tree seedlings, the total quantity of isotopes fixed by roots into any one metabolite is compared under air and nitrogen, then it is only malic acid which shows a regular response to the different treatments. This stresses, therefore, the key position of malic acid in the metabolism deriving from the $C^{14}O_2$ -fixation of roots. Also, the dependence of the total amount of labelled malic acid on the availability of oxygen decreases with the increasing flooding-tolerance of the species used.

For all substances other than malate, it is not the total counts fixed in any particular metabolite that was found to be of significance, but rather the percentages of the total counts detected in any one substance under the different treatments. Using only the roots of tree seedlings, it seems that the turnover of their $C^{14}O_2$ -fixation products is never rapid, as the label is only slowly dispersed into the general metabolic pool. Attention should also be drawn to the fact that higher percentages of labelled succinic acid were always detected after fixation under nitrogen than after fixation under air. This may indicate that in tree seedlings an anaerobic dehydrogenation of NADH, leading to the reduction of fumarate to succinate, could provide energy in the form of ATP, such as Bueding (1962) discovered in the muscles of the marworm Ascaris sp.

A further general characteristic of the $C^{14}O_2$ -fixation of tree roots seems to be that under anaerobic Conditions, the transamination reactions are of more importance in the turnover of labelled metabolites than during similar $C^{14}O_2$ -fixation under air. In particular, when fixation takes place under oxygen, the labelling in aspartic acid and alanine are still further decreased, suggesting that their formation is independent on a sufficient supply of NADH and ATP.

Apart from these considerations, the $C^{14}O_2$ -fixation in the roots of tree seedlings seems to show that there are certain regularities in the distribution pattern of some metabolites, which appear to be linked to the

various experimental flooding conditions. Four days of flooding seem to reduce the overall turnover rate of organic acids which leads to an increase in the percentages of labelled malic acid, while C^{14} incorporation into citric and succinic acids decreases. Further, the sum of the percentages of labelled organic acids increases due to the flooding pretreatment. Additional nutrients during a prolonged flooding period, however, decrease the sum of the percentages of labelled organic acids on the one hand, and increases the sum of the percentages of labelled amino acids on the other, in all the species used. This seems to suggest that a prolonged pretreatment with Hoagland solution enhances the nitrate assimilation processes in the roots of tree seedlings. Thus, from these data, the important discovery emerges that under nitrogen, additional nutrients during a prolonged pretreatment enhance the percentages of labelled amino acids - or just even the sum of the percentages of labelled glutamic acid, alanine and citrulline. These increases are greatest in flood-tolerant species. In other words, the increase here in the flooded over the unflooded plants is directly proportional to the flooding-tolerance of the species. Hence, it seems that under nitrogen, the nitrate assimilation processes after a pretreatment with Hoagland solution are enhanced more in the flood-tolerant species than in the non-tolerant tree seedlings.

From the foregoing consideration one might be able to infer that after a prolonged flooding treatment with Hoagland solution, the increase in labelling of amino

acids is due not just to the application of additional nutrients, but also to the waterlogged soil conditions during the pretreatment. A pretreatment of flooding for four days with distilled water revealed that under nitrogen, the percentages of labelled amino acids actually decrease in the flood-intolerant tree seedlings; the converse finding, however, was obtained with the flood-tolerant species Pinus contorta.

Thus, in contrast with nutrient solution, a completely different picture seems to emerge after a prolonged pretreatment with distilled water. Under nitrogen, the sum of the percentages of labelled amino acids as well as the individual percentages of alanine, aspartic and glutamic acids decrease only in the flood-tolerant species, and not in the intolerant tree seedlings. It is of particular note that when tree seedlings, which have been flooded for a long period with distilled water are compared with their unflooded control plants, the decrease in labelling of glutamic acid (under nitrogen) is inversely proportional to the flooding-tolerance of the species. In summary, therefore, flooding with distilled water seems to reduce under anaerobic conditions the overall turn over rate of amino acids, or the deproteinization in flood-tolerant species. The converse is true of intolerant species.

Now that the various findings and methods of this study into the effects of waterlogged soil conditions on tree seedlings have been treated separately, a general evaluation of them is necessary. The growth tests have suggested that the oxygen flux to the roots via the shoots is not of great significance in mature trees. However, the morphological differences observed in the course of experimental flooding provided a sequence for the relative flood-tolerance in all the different species and provenances used. The

repetition of this sequence was striking (see Table D). This Table also shows the clear differences between flood-tolerant and non-tolerant species as regards the total $C^{14}O_2$ -fixation of the roots of tree seedlings; and the relative labelling of certain metabolites after a $C^{14}O_2$ -fixation of roots. Moreover, investigations into the accumulation of non-toxic metabolites in the roots of tree seedlings or in the rising sap of mature trees, under conditions of partial or complete anoxia, seems to be of relevance in the search for additional possibilities for determining the relative flooding-tolerance of different tree species and varieties.

The methods used in this study are already described in the literature, and were chosen according to the apparatus available in the Department. Experimental flooding in the glasshouse was largely preferred to the collection of samples in the field. Apart from practical reasons, this was because in the glasshouse it was possible to operate a 16-hour day, eliminate the influence of wind, and maintain regular temperatures. It is true that experiments in a glasshouse are often viewed with scepticism, the behaviour of plants in the field being to some extent different. Notwithstanding this, the growth tests with tree seedlings showed that a flooding-tolerance classification obtained in a glasshouse is related to the behaviour of different species in the field, i.e. the natural habitats, the origin of the seeds, and growth in the experimental garden.

Table B. Summary by ranking of tree seedling performance comparing shoot growth after flooding with the various metabolic tests employed.

I. Sequences.	Experimental flooding shoot growth test	Unflooded tree seedlings; Dependence on oxygen availability; Total C ¹⁴ O ₂ fixation of roots	Distilled water flooded:unflooded Percentages of labelled glutamic acid under N ₂	Hoagland soln.:distilled water Percentages of labelled amino acids under N ₂ and citrulline under N ₂			
Pinus contorta	1	1	1	1	1		
Alnus incana	2	2	2	2	2		
Larix laricina	3	3	3	3	3		
Picea mariana	4	4	4	4	4		
Picea sitchensis	5	5	5	5	5		
Pinus sylvestris	6	6	6	6	6		
Larix europea	7	7	-	-	-		
Original data see Tables 1.5 & 1.18.	Tab.1.2,1.3, 1.5 & 1.18.	Tab. 3.1.	Tab. 3.7.	Tab. 3.12.	Tab. 3.14.		
- not determined.							
The significant correlation between the above ranking is obvious.							
II. Distinctive pattern for flood-tolerant and non-tolerant species.							
Species	Formation of adventitious roots	Influence of additional nutrients on total C ¹⁴ O ₂ fixed/CO ₂ evolved	Dependence on oxygen supply after flooding with Hoagland solution on glutamic and aspartic acids	P e r c e n t a g e s o f: labelled citric acid under N ₂ labelled amino acids under N ₂ labelled aspartic acid under N ₂ of: labelled alanine under N ₂ tyrosine under air			
Pinus contorta	3	1	1	1	2	2	1
Alnus incana	1	3	1	3	1	1	3
Larix laricina	2	2	1	2	3	3	2
Picea mariana	4	4	1	6	4	6	6
Picea sitchensis	5	6	4	4	6	5	5
Pinus sylvestris	6	5	4	5	5	4	4
Original data see Tables 1.6, & 1.7.	Tables 2.9 & 2.22.	Table 3.10	Table 3.3.	Tab.3.4	Tab.3.5	Tab.3.6.	Tab.3.8.

One might well ask why the number of samples for most experiments described in this study was so limited. While the selection of the plant material was done at random, it was nonetheless striking to find in fact corresponding sequences for the relative flooding-tolerance of different species and provenances. Even when using such different methods as the $C^{14}O_2$ -fixation and growth rates the same sequence of behaviour was found. This would seem to justify the taking of a small sample number. More than that, the use of a large number of different species and provenances for different experiments, rather than making a thorough examination of just a few characteristics of a small number of species, could be of greater biological value in a preliminary investigation into an ecological problem. This is because the intensive examination of a small number of species is more likely to yield only differences typical for different species, but bearing no relation to the response in environmental conditions, which had to be investigated.

It seems, therefore, that we are now closer to establishing a test for flooding-tolerance in trees. Some evidence was found that the ability of flood-tolerant mature trees to resist the stress of a surplus watersupply may be linked to the characteristic not only of producing certain metabolites in their roots in preference to others, but also of translocating anaerobic respiration products from the roots to the aerial sections of the trees. It was also discovered that morphological changes in tree seedlings under experimental flooding are accompanied by

quantitative changes in the distribution pattern of the $C^{14}O_2$ -fixation products of their roots. Yet the dependence of the total $C^{14}O_2$ -fixation of the roots of unflooded tree seedlings on oxygen availability can already be used to predict the relative flooding-tolerance of different species and provenances.

It is to be hoped that subsequent investigations of a biochemical nature into the particular problem of establishing a test for the flooding-tolerance of trees, by using higher sample numbers, examining more species of different provenances, using the same or different methods, such as the polarographic techniques etc., will be of help in confirming the findings of this study, as well as bringing a new dimension into ecology from the field of molecular biology.

SUMMARY

The essential object of this study has been to carry out some basic preliminary investigations necessary in the quest for an eventual metabolic test of flooding-tolerance that can be used with tree seedlings. There are immense practical advantages to be gained by being able to determine at an early stage whether or not mature trees of any species or provenances will be resistant to the usually detrimental effects of flooding.

The relative flooding-tolerance of tree seedlings was ascertained by observing the differing morphological behaviour during an experimental flooding period in the glasshouse:-
1. When the growth-rates of tree seedlings were compared after the water table had been lowered following a half-year flooding period, a primary classification of different species and different provenances was possible.

2. During the experimental flooding it was found that in less tolerant species, the onset of dormancy of shoot growth was accelerated.

3. Pinus contorta seedlings of different provenances which revealed a higher root:shoot ratio seemed to have a better capability to withstand waterlogged conditions than other varieties.

It is generally supposed there are two possible adaptation mechanisms in plants known to be resistant to flooding, which may enable them to maintain a living root system in waterlogged soil(s):-

A. Morphological adaptations facilitating gas exchange between root and shoot:

1. It was found that seedlings which form new roots visible on the soil surface were more successful in withstanding the stress of a surplus water supply.

2. The growth test with seedlings of different heights and ages suggested that the oxygen flux to the roots via the shoot was not the only factor associated with the survival of mature trees.

3. The diffusion of methane to the shoot via root was higher in flood-tolerant species than in flood-intolerant species.

B. Metabolic properties associated with the tolerance of anoxia by root tissues:

1. A non-toxic anaerobic respiration product increased the ability to resist a period of anoxia.

(i) It was found that during the first few days of flooding, the

glycerol content increased rapidly in Alnus incana roots. This production of glycerol can continue without limit in the absence of oxygen and provides an alternative to ethanol as the end product of anaerobic respiration.

(ii) In the rising birch sap (Betula pubescens), the malic acid concentration was found to be circa 100 times greater than that of ethanol, and on a wet site, the malic acid content was twice as high as on a dry site. It would thus appear possible that roots respiring anaerobically and producing mainly malate, repay their oxygen debt by transporting the malic acid upwards in the rising sap.

2. Changes in the $C^{14}O_2$ -fixation of roots can be used as indicator of relative flooding-tolerance in tree seedlings:

(i) In flood-tolerant species, the total $C^{14}O_2$ -fixation of roots was less dependent on oxygen availability than it was in flood-intolerant species.

(ii) The net gain of CO_2 in roots of flood-tolerant species was higher after a prolonged treatment with Hoagland solution than with distilled water, and the converse was found for flood-intolerant species.

(iii) When tree seedlings have been flooded for a prolonged period, the R.Q. of the roots increased above one in flood-intolerant species only.

(iv) The dependence of the total amount of labelled malic acid on oxygen availability decreased with the increasing flooding-tolerance of the species used.

(v) After a prolonged flooding treatment with distilled water the sum of the percentages of lable fixed into amino acids under nitrogen decreased in the flood-tolerant species only. In the non-tolerant tree seedlings there was an increase in the lable found in this fraction. In particular, the labelling of glutamic acid was inversely proportional to the flooding-tolerance of the species.

(vi) Under nitrogen, and after a prolonged treatment with distilled water, the percentages of labelled citric acid increased in flood-tolerant species only.

(vii) Under nitrogen, additional nutrients during a prolonged flooding treatment enhanced the percentages of labelled amino acids. This increase was in direct proportion to the flooding-tolerance of the tree seedlings used.

All these findings point to the consideration that not only

growth tests with tree seedlings, but also attention to metabolic changes in roots of tree seedlings and in the rising sap of mature trees might be of help in establishing a simple flooding-tolerance test for trees.

REFERENCES

- ARMSTRONG, W. (1964) Oxygen diffusion from the roots of some British bog plants. Nature (Lond.), 204, 801-802.
- (1967) The relationship between oxidation reduction potentials and oxygen diffusion levels in some waterlogged soils. J. Soil Sci., 18, 27-34.
- (1968) Oxygen diffusion from the roots of woody species. Physiologia Pl., 21, 539-543.
- BARTHELMESS, A. (1972) Wald - Umwelt des Menschen. Dokumente zu einer Problemgeschichte von Naturschutz, Landschaftspflege und Humanökologie. Verlag Alber, Freiburg/München.
- BEEVERS, H. (1961) Respiratory metabolism in plants. Row, Peterson Biological Monographs, New York.
- BENSON, A.A., BASSHAM, J.A., CALVIN, M., GOODALE, T.C., HASS, V.A., and STEPKA, W. (1950) The path of carbon in photosynthesis. V. Paper chromatography and radiography of the products. J. Amer. Chem. Soc., 72, 1710.
- BERGMEYER, H.U. (1963) Methods of enzymatic analysis. London.
- BERRY, L.J. (1949) The influence of oxygen tension on the respiratory rate in different segments of onion roots. Cell and Comp. Physiol., 33, 43-46.
- BETZ, A. (1957) Zur Atmung wachsender Wurzelspitzen in Stickstoff und hochprozentiger Sauerstoffatmosphäre. Die Pasteur Reaktion. Planta 50, 122-143.
- BEYER, E.M. and MORGAN, P.W. (1970) A method for determining the concentration of ethylene in the gas phase of vegetative plant tissues. Plant Physiol., 46, 352-354.
- von BRAND, T. (1966) Biochemistry of parasites. London.
- BRYANT, C. (1971) The biology of respiration. London.
- BUNDING, E. (1962) Comparative aspects of carbohydrate metabolism. Federation Proc., 21, 1039-1046.
- CALVIN, M., BASSHAM, J.A., BENSON, A.A., LYNCH, V.H., OUELLET, C., SCHOU, L., STEPKA, W. and TOLBERT, N.E. (1951) Carbon dioxide assimilation in plants. In: Symp. Exp. Biol. V, 284-306.

- CARLES, J., SCHNEIDER, A. and LACOSTE, A.M. (1958) Contribution à l'étude chromatographique des principaux acides organiques. Bull. Soc. Chim. Biol., 40, 221-232.
- ČIRKOVA, T.C. (1968) Features on the oxygen supply of roots of certain woody plants in anaerobic conditions. Fiziol. Rast., 15, 565-568.
- CRAWFORD, R.M.M. (1966) The control of anaerobic respiration as a determining factor in the distribution of the genus Senecio. J.Ecol., 54, 403-413.
(1967) Alcohol dehydrogenase activity in relation to flooding tolerance in roots. J. exp. Bot., 18, 458-468.
(1972) Physiologische Ökologie: Ein Vergleich der Anpassung von Pflanzen und Tieren an sauerstoffarme Umgebung. Flora, 161, 209-223.
and TYLER, P.D. (1969) Organic acid metabolism in relation to flooding tolerance in roots. J.Ecol., 57, 237-246.
- DUBININA, L.M. (1962) Metabolism of roots under various levels of aeration. Soviet Plant Physiology, 8, 314-322.
- ERICKSON, A.E. (1965) Short term oxygen deficiencies and plant responses. Amer. Soc. Agr. Eng. (St. Joseph, Mich.), Conf. Proc. (Drainage for efficient crop production) 11-23.
- FULTON, J.H. and ERICKSON, A.E. (1964) Relation between soil aeration and ethyl alcohol accumulation in xylene exudate of tomatoes. Soil Sci. Soc. Amer. Proc., 28, 610-614.
- FULTON, J.H., ERICKSON, A.E. and TOLBERT, N.E. (1964) Distribution of C¹⁴ among metabolites of flooded and anaerobically grown tomato plants. Agron. J., 56, 527-529.
- GILL, C.J. (1970) The flooding tolerance of woody species. A review. Forestry Abstracts, 31, 671-688.
- GILMOUR, D. (1961) The biochemistry of insects. Academic Press, London and New York.
- GREENWOOD, D.J. (1967) Studies on the transport of oxygen through the stems and roots of vegetable seedlings. New Phytol., 66, 337-347.
- DEVAUX, H. (1899) Asphyxie spontanée et production d'alcool dans les tissus profonds des tiges ligneuse poussant dans les condition naturelles. C. r. hebdom. Acad. Sci. Paris, 128, 1346-1349.

- GREENWOOD, D.J. (1969) Effect of oxygen distribution in the soil on plant growth. In: Root growth (Proc. 15th Easter School in Agric. Sci., Nottingham). Ed. W.J. Whittington.
- HAGEMAN, R.H. and FLESHER, D. (1960) The effect of an anaerobic environment on the activity of alcohol dehydrogenase and other enzymes of corn seedlings. Arch. Biochem. Biophys., 87, 203-209.
- HARTMANN, R.T. and BROWN, D.L. (1966) Methane as a constituent of the internal atmosphere of vascular hydrophytes. Limnology and Oceanography, 11, 109-112.
- (1967) Changes in internal atmosphere of submerged vascular hydrophytes in relation to photosynthesis. Ecol., 48, 252-258.
- HAUSMANN, W. (1952) Amino acid composition of crystalline inorganic pyrophosphates from baker yeast. J. Amer. Chem. Soc., 74, 3181.
- HAWKER, L.E. and LINTON, A.H. (1971) Micro-organisms. Edward Arnold Ltd.
- HENSHAW, G.G., COULT, D.A. and BOULTER, D. (1962) Organic acids of the rhizome of Iris pseudacorus L. Nature (Lond.), 194, 579-580.
- HIGGINS, H. and von BRAND, T. (1966) Separation of lactic acid and some Krebs cycle acids by thin-layer chromatography. Analyt. Biochem. 15, 122-126.
- HOFFMANN, G. (1972) Wachstumsrhythmik der Wurzeln und Sprossachsen von Frostgehölzen. Flora 161, 303-319.
- HOOK, D.D., BROWN, C.L. and KORMAIK, P.P. (1971) Inductive flood tolerance in swamp tupelo (Nyssa sylvatica var. biflora (Walt.) Sarg.). J. exp. Bot., 22, 78-89.
- HURIKARI, O. (1954) Experiments on the effect of anaerobic media upon birch, pine and spruce seedlings. Commun. Inst. Forest. Fenn. 42, 1-13,
- JACKSON, W.T. (1955) The role of adventitious roots in recovery of shoots, following flooding of the original root systems. Amer. J. Bot., 42, 816.
- (1956) Flooding injury studied by approach-graft and split root system techniques. Amer. J. Bot., 42, 816-819.
- JAMES, W.O. (1951) Plant respiration. Oxford.

- KALELA, E.K. (1950) Männikoiden ja kuusikoiden juurisuhteista it. Acta. Forest. Fenn., 57, 1-68.
- KANDLER, O. (1958) Zur Frage einer aeroben Gärung in Wurzelmeristemen. Planta, 51, 544-546.
- KARLSON, P. (1966) Kurzes Lehrbuch der Biochemie. Thieme Verlag Stuttgart.
- KOLLÖFFEL, C. (1968) Activity of alcohol dehydrogenase in the cotyledon of peas germinated under different environmental conditions. Acta Bot. Neerl., 17, 70-77.
- KRAMER, P.J. (1957) Some effects of various combinations of day and night temperatures and photoperiod on the height of loblolly pine seedlings. Forest. Sci., 3, 45-55.
- (1969) Plant and soil water relationships: A modern synthesis. McGraw Hill. Series in organismic biology. New York.
- and JACKSON, W.T. (1954) Causes of injury to flooded tobacco plants. Plant Physiology, 29, 241-245.
- and KOZLOWSKI, T.T. (1960) Physiology of trees. McGraw Hill. New York.
- LAVARENNE-ALLARY, S. (1966) Croissance rythmique de quelques espèce de chêne cultivées en chambres climatisées. C. r. heb. Seanc. Acad. Sa. Paris 262 D, 358-361.
- LEYTON, L. and ROUSSEAU, L.Z. (1957) Root growth of tree seedlings in relation to aeration. In: The physiology of forest trees (Ed. K.V. Thimann). Ronald Press, New York.
- MAZELIS, M. and VENNESLAND, B. (1957) Carbon dioxide fixation into oxalacetate in higher plants. Pl. Physiol., Lancaster, 32, 591-600.
- MCMANNON, M. and CRAWFORD, R.M.M. (1971) A metabolic theory of flooding tolerance: The significance of enzyme distribution and behaviour. New Phytol. 70, 299-306.
- OSBORNE, D.J. (1968) Ethylene as a plant hormone. Plant growth regulators, S.C.J. Monograph 31, 236-249.
- POEL, L.W. (1953) Carbon dioxide fixation by barley roots. J. Exp. Bot., 4, 157-163.

- PONNAMPERUMA, F.N., ESTRELLA MARTINEZ and TERESITA LOY (1966) Influence of redox potential and partial pressure of carbon dioxide on pH values and the suspension effect of flooded soils. Soil Science, 101, 421-431.
- PRATT, H.K. and GOESCHL, I.D. (1951) Physiological roles of ethylene. A.Rev.Pl.Physiol., 26, 363-368.
- REID, D.M. and CROZIER, A. (1971) Effects of waterlogging on the gibberline content and growth of tomato plants. J. Exp. Bot., 22, 39-48.
- SMITH, K.A. and SCOTT RUSSELL, R. (1969) Occurrence of ethylene and its significance in anaerobic soil. Nature (Lond.), 222, 769-771.
- SPURR, S.H. (1964) Forest Ecology. Ronald Press Co. New York.
- SWEELY, C.C., BENTLEY, R., MAKITA, M. and WELLS, W.W. (1963) Gas liquid chromatography of trimethyl silyl derivatives of sugars and related substances. J. Amer. Chem. Soc., 85, 2497.
- THOMAS, M.T., RANSON, S.L. and RICHARDSON, J.A. (1956) Plant Physiology (4th edit.) p.236. J. & A. Churchill, London.
- VERETENNIKOV, A.V. (1968) (Physiological foundations of the resistance of woody plants to temporary excess moisture in the soil.) Izdatel'stvo 'Nauka', Moscow.
- VLAMIS, J. and DAVIS, A.R. (1944) Effects of oxygen tension on certain physiological responses of rice, barley and tomato. Pl. Physiol., 19, 33-51.
- WAGER, H.G. (1961) The effect of anaerobiosis on acids of the tricarboxylic acid cycle in peas. J. exp. Bot., 12, 34-46.
- WALKER, L.C. (1967) Effects of water level and fertilizer combinations on loblolly and slash pine seedlings. Tree Plant Notes, 18, 10-12.
- WILLIAMS, W.T. and BARBER, D.A. (1961) The functional significance of aerenchyma in plants. Symp. Soc. Exptl. Biol., 15, 132-144.
- WOOD, H.G. (1951) A consideration of some reactions involving carbon dioxide fixation. Symp. Soc. Exp. Biol., 5, 9-28.

ZUSAMMENFASSUNG

Die Hauptaufgabe dieser Studie war es, elementare Untersuchungen durchzuführen, um ein stoffwechselphysiologisches Testverfahren für die Überflutungstoleranz der Bäume zu finden. Die Verwendung von Baumsämlingen verschiedener Arten mit bekannten Herkunftsorten ist hierfür von grossem praktischen Vorteil.

Durch Überflutungsexperimente im Gewächshaus wurde die relative Überflutungstoleranz der Baumsämlinge auf Grund ihres morphologisch unterschiedlichen Verhaltens bestimmt:

1. Wurde der Wasserspiegel nach einem halben Jahr Überflutung gesenkt, so war eine erste Klassifizierung der verschiedenen Arten von verschiedenen Herkunftsorten möglich.
2. Durch experimentelles Überfluten trat in weniger Überflutungstoleranten Arten früher ein Ruhezustand des Sprosswachstums ein.
3. Ein höheres Wurzel-zu-Spross Verhältnis bei Sämlingen der Art Pinus contorta von verschiedenen Herkunftsorten, zeigte jene Varianten an, welche besser für überflutete Böden geeignet sind. Im allgemeinen werden zwei Anpassungsmechanismen erwogen, wodurch überflutungstolerante Pflanzen ein lebendes Wurzelsystem in staunassen Böden beibehalten:

A. Morphologische Anpassungen, welche den Gasaustausch zwischen Wurzeln und Spross erleichtern:

1. Sämlinge, welche neue, an der Bodenoberfläche sichtbare Wurzeln bilden, waren erfolgreicher, ein Übermass an Wasserversorgung zu überstehen.
2. Überflutungsversuche mit Sämlingen verschiedener Sprosshöhe und verschiedenen Alters liessen vermuten, dass die Sauerstoffversorgung der Wurzeln durch den Spross nicht der einzige Faktor sei, der für das Überleben von Bäumen wichtig ist.
3. In Überflutungstoleranten Arten war die Methandiffusion von den Wurzeln in den Spross grösser als in den intoleranten Arten.

B. Stoffwechselphysiologische Eigenschaften, die im Zusammenhang mit der Toleranz der Wurzeln gegenüber sauerstoffarmer Umgebung stehen:

1. Ein nicht toxisches anaerobes Respirationsprodukt steigert die Resistenz gegenüber periodischer Sauerstoffarmut:
 - a. Während der ersten Tage der Überflutung stieg der Glyzerin-gehalt in den Wurzeln von Alnus incana rasch an. Die Glyzerinproduktion ist nicht sauerstoffabhängig und kann daher als eine Alternative zu einer anaeroben Äthanolproduktion angesehen werden.
 - b. Im aufsteigenden Birkensaft (Betula pubescens) war die Äpfel-

säurekonzentration zirka 100 mal grösser als die des Äthanolis, und in Bäumen von einem nassen Standort war die Äpfelsäurekonzentration zweimal so hoch wie in jenen von einem trockenen Standort. Es scheint daher möglich, dass Wurzeln, die anaerob atmen und hauptsächlich Malat produzieren, ihre Sauerstoffschuld durch einen Abtransport der Äpfelsäure im aufsteigenden Saft begleichen.

2. Unterschiede in der $C^{14}O_2$ Fixierung der Wurzeln können als Indikator für die Überflutungstoleranz von Baumsämlingen benutzt werden:

a. In Überflutungstoleranten Arten war die gesamte $C^{14}O_2$ Fixierung der Wurzeln weniger abhängig von dem Vorhandensein von Sauerstoff als in den Überflutungsuntoleranten Arten.

b. Der Nettogewinn an CO_2 der Wurzeln von Überflutungstoleranten Arten war nach einer verlängerten Behandlung mit Hoagland's Nährlösung höher als mit destilliertem Wasser und das Gegenteil wurde für die Überflutungsuntoleranten Arten gefunden.

c. Waren Baumsämlinge für eine verlängerte Periode überflutet, so stieg der respiratorische Quotient in den Wurzeln der untoleranten Arten über eins an.

d. Die Abhängigkeit des Gesamtbetrages an markierter Äpfelsäure gegenüber dem Vorhandensein von Sauerstoff nahm mit zunehmender Überflutungstoleranz der Arten ab.

e. Nach einer verlängerten Überflutungsvorbehandlung mit destilliertem Wasser nahm unter Stickstoff die Summe der Prozentsätze an markierten Aminosäuren nur in den Überflutungstoleranten Arten ab. In den untoleranten Baumsämlingen war ein Anstieg der Markierung in dieser Fraktion zu beobachten. Besonders bemerkenswert ist, dass die Markierung von Glutaminsäure umgekehrt proportional zu der Überflutungstoleranz der Arten war.

f. Nach einer verlängerten Vorbehandlung mit destilliertem Wasser und unter Stickstoff nahm nur in den Überflutungstoleranten Arten die Markierung von Zitronensäure zu.

g. Eine verlängerte Überflutungsperiode mit Hoagland's Nährlösung steigerte unter Stickstoff die Prozentsätze an markierten Aminosäuren. Dieser Anstieg war direkt proportional zu der Überflutungstoleranz der Baumarten. All diese Ergebnisse unterstreichen, dass nicht nur Wachstumsversuche mit Baumsämlingen sondern auch stoffwechselphysiologische Unterschiede in den Wurzeln von Baumsämlingen und im aufsteigenden Saft von Bäumen von Nutzen für einen einfachen Überflutungstoleranztest der Bäume sein könnten.



